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THE LATERAL BLOOD SUPPLY OF PRIMITIVE ELASMOBRANCH FISHES

BY

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THE LATERAL BLOOD SUPPLY OF PRIMITIVE ELASMOBRANCH FISHES

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INTRODUCTION

In all present-day elasmobranch fishes a lateral artery and a lateral vein connect pectoral and pelvic areas. These vessels occupy a position at the base of the lateroventral muscle bundle, and if seen from the coelomic cavity lie under the peritoneum. In this view, the vein appears as an unusually large vessel and the artery, which is much smaller in caliber, lies deeper in the body wall. In the region of the trunk these vessels run a more or less monotonous course, changing only slightly in diameter. At the pectoral girdle, however, their relations to other vessels attract our attention. Especially is this true of the lateral artery which through its connection with the coracoid artery bears a most remarkable relationship to the complex hypobranchial system of vessels..

THE LATERAL ARTERY AND ITS RELATIONS

T. Jeffery Parker (1886) in his monograph on the "Blood Vessels of *Mustelus antarcticus*" after referring to the lateral artery as a branch of the "hypobranchial" says that the hypobranchial artery arises from the subclavian and passes "forward." The relation of the hypobranchial to the subclavian, described by Parker, seems to be characteristic of all the more specialized elasmobranchs. For example, in a ray like *Dasyatis dipterura* Parker's hypobranchial, which I have designated as the coracoid (Daniel, 1922, fig. 161, *co.a.*), appears to arise from the large subclavian artery, and it seems a reasonable assumption that it carries blood forward. At any rate, one would not expect any of the blood from the coracoid in *Dasyatis* to reach the pectoral fin; for the chief source of blood to the pectoral area is through the large subclavian artery.

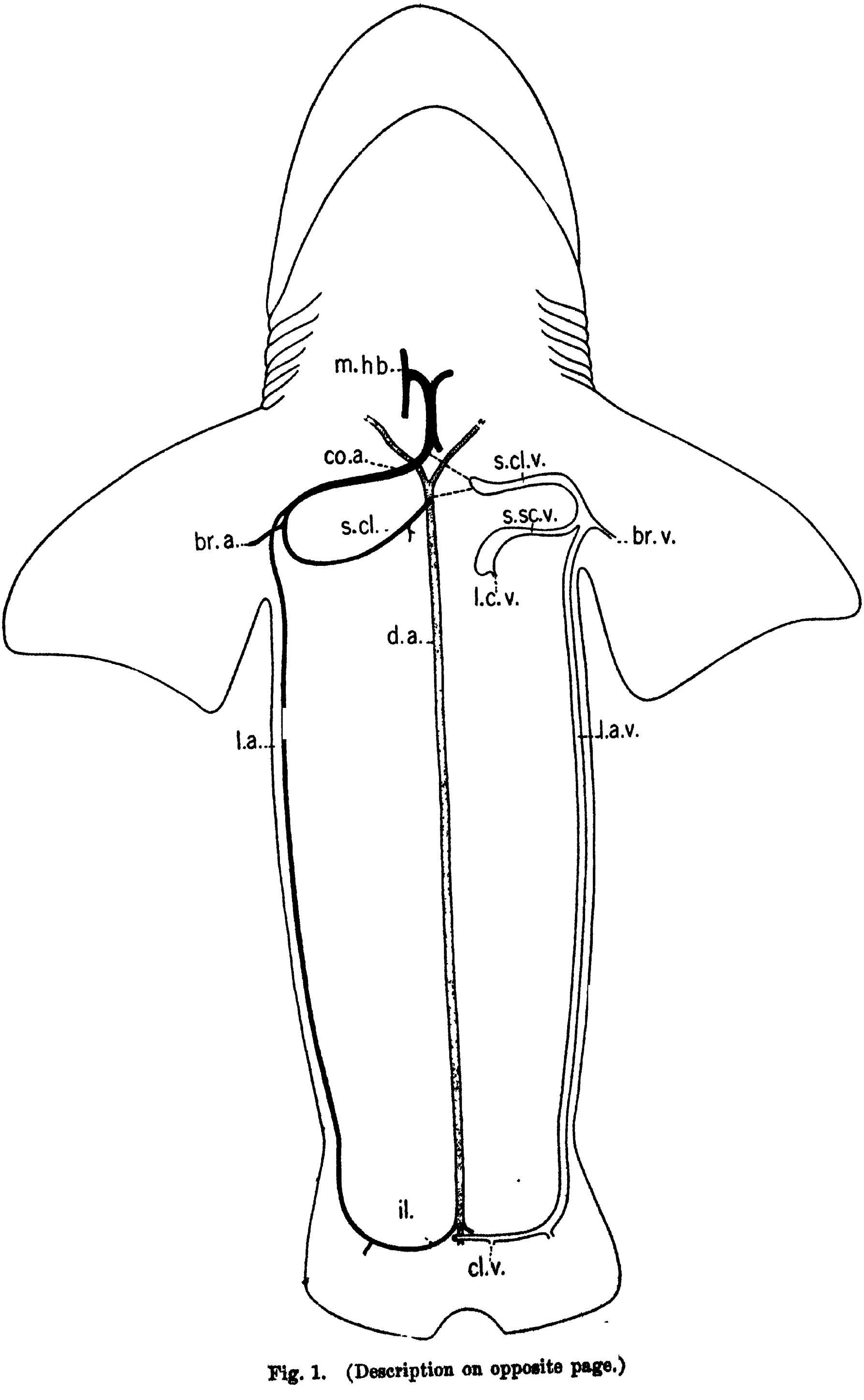

Fig. 1. (Description on opposite page.)

In generalized elasmobranchs the case is different; for in these the coracoid, the "hypobranchial" artery of Parker, does not arise from the subclavian, that is, it is not an ascending vessel. Further, the union which the coracoid makes with the subclavian indicates that the subclavian joins it only secondarily. In generalized elasmobranchs the lateral artery (fig. 1, *l.a.*) appears to be the direct posterior continuation of the coracoid to the pelvic area (Daniel, 1925).

With the above remarks in mind I wish to consider the main arterial line to the paired fins as composed of two segments, (1) the coracoid artery and (2) the lateral artery, and to show the relation of this coracolateral line to the true hypobranchial arteries and to the subclavian artery.

In *Heptanchus maculatus* the coracoid arteries (fig. 1, *co.a.*) arise as right and left vessels from true median hypobranchial arteries (*m.hb.*) between commissurals two and three, or three and four, and then pass posteriorly to join in the midventral line as a single large trunk. The single trunk, after continuing posteriorly three or four segments farther, bifurcates and its halves swing outward and upward along the coracoid cartilages. In the pectoral region after giving off metapterygial and a well developed brachial branch (*br.a.*) to the pectoral fin, each coracoid continues as the lateral artery.

As to the direction of the flow of blood in the coracoids of *Heptanchus* there can be little doubt but that it is posterior, for it is principally from this source that the pectoral fin receives its supply.

Although the subclavian (*s.cl.*) makes connection with the coracoid artery (*co.a.*) this connection is of such a nature that it would be unsafe to assume that the latter vessel arises from the former, for the coracoid is a stout trunk while the subclavian is a smaller vessel. Again, the branching of the subclavian suggests that its union with the coracoid may have taken place secondarily. In general the subclavian in *Heptanchus* branches much like the segmental arteries. But some of its branches may ramify toward the dorsal aorta (*d.a.*) indicating that the flow of blood in these branches might even be away from the pectoral area. Further, the distal segment of the subclavian uniting with the coracoid may, as I have shown previously (fig. 149, 1922), be larger in caliber than the median segment of this artery.

Fig. 1. Lateral blood supply in *Heptanchus maculatus*, ventral view. *br.a.*, brachial artery; *br.v.*, brachial vein; *cl.v.*, cloacal vein; *co.a.*, coracoid artery; *d.a.*, dorsal aorta; *il.*, iliac artery; *l.a.*, lateral artery; *l.a.v.*, lateral abdominal vein; *l.c.v.*, lateral cutaneous vein; *m.hb.*, median hypobranchial artery; *s.cl.*, subclavian artery; *s.cl.v.*, subclavian vein; *s.sc.v.*, subscapular vein.

In specimens in which this is marked this distal segment looks much like a regular branch of the coracoid proper.

From the diminutive size of the subclavian and the character of its branching and its union with the coracoid in a simple type like *Heptanchus maculatus*, I have come to the opinion that the subclavian arose in the elasmobranchs as a segmental branch of the dorsal aorta and that this branch upon the localization of the pectoral fin joined the area of the fin secondarily by anastomosing with a branch coming off from the coracolateral vessel.

If this hypothesis be correct, it would throw light on the enigma of why, in more specialized elasmobranchs, arterial and venous trunks which supply the pectoral area are so widely separated. As a rule arterial and venous trunks of a given region run parallel and in relatively close proximity to each other. In the area under consideration the dorsal aorta which, through the subclavian in higher elasmobranchs, supplies the pectoral area with blood lies under the spinal column and in the mid-dorsal line. The lateral abdominal vein, on the other hand, which relieves this area of blood, lies in the lateroventral body wall. The answer to the enigma then would be that the present condition in which the pectoral fin receives its arterial supply from so great a distance has been derived secondarily; that originally the supply to the pectoral area was from the coracolateral artery which ran parallel with and in close proximity to the lateral abdominal vein.

ARTERIAL SUPPLY TO THE PECTORAL AREA IN PRIMITIVE ELASMOBRANCHS

It is of course unlikely that we shall ever know much concerning the circulatory system of ancient elasmobranch fishes, since this system is subject to rapid decay. But it seems to me not unlikely that, if we knew the arterial system in a type still more ancient than *Heptanchus*, say even in *Cladoselachus* where the pectoral fin had a greater basal expanse, we should probably find that the arterial supply to the pectoral area was not from the subclavian artery as it is in present-day vertebrates but was wholly from the coracolateral artery.

It may be argued that the coracoids are recent vessels. In fact we know that they are more recent than the embryonic aortic arches, for they arise from derivatives of these arterial arches. But they may be more recent than the embryonic aortic arches and still be practically as old as gills of the elasmobranch type. What the state of the paired fins was at the time of the breaking of the embryonic aortic arches into afferent and efferent components with the concomitant development of such tributaries as the coracoid we, of course, do not know. If we assume that paired fins were then only slightly modified lateral folds and that the coracoids with their extensions, the lateral arteries, supplied the larger part or the whole of these folds, then the coracoid vessels would have been of great importance in connecting the lateral arteries with their sources of supply in the hypobranchial region.

THE LATERAL VEIN AND ITS RELATIONS

There is evidence that some of the tributaries of the lateral abdominal vein in *Heptanchus* are more primitively arranged than in higher elasmobranchs. Thus in the region of the pelvic area the cloacal and femoral veins (Daniel, 1922, fig. 177, *cl.v.*, *f.v.*) enter the lateral abdominal vein (*l.a.v.*) as distinct and separate, myomeric vessels, while in other and more specialized sharks they fuse and enter as a single trunk.

In the pectoral region the lateral abdominal vein bears a peculiar relation to other vessels and may be regarded as of greater importance in *Heptanchus* than it is in most other elasmobranchs; for it receives a relatively larger venous supply. In addition to draining the segment of the pectoral girdle through the subscapular vein (fig. 1, *s.sc.v.*; cf. also Daniel, 1918, fig. 1), it also receives the supply of the lateral cutaneous vein (*l.c.v.*) which empties into the dorsal part of the subscapular. It does not follow, however, that, because the subscapular supply to the lateral vein is large, the vein itself is primitive. In fact on the assumption of a lateral fin-fold we should expect the pectoral girdle and its present associated vein, the subscapular, to be less ancient than the fin-fold itself. It may be that the subscapular vein has captured the lateral cutaneous supply by reason of its growing importance just as the postcardinal of *Scyllium* (O'Donoghue, 1914, p. 438) or *Mustelus* (Parker, 1886, pp. 715–716) now captures it. I have shown in a former paper (1918, *loc. cit.*) that *Squalus sucklii* may use either the postcardinal or lateral abdominal vein for the capture of the cutaneous supply. In the case of *Squalus sucklii* the connection between the lateral cutaneous vessel and the postcardinal appears to be of recent origin. In *Heptanchus* the relation of the lateral cutaneous to the lateral abdominal veins through the subscapular is possibly as old as the pectoral girdle; while in the more remote ancestors of *Heptanchus* it is likely that the lateral cutaneous vein, provided such a vein then existed, was relieved in part by metameric veins, one of which represented the now well established subscapular vein.

SUMMARY

My concept of the lateral blood supply in the early elasmobranch fishes may be summarized as follows. Blood for the area of the paired fins, perhaps soon after its aeration in the gills, passed from the ventral loops of the efferent-collectors through median hypobranchial arteries into right and left coracoids and on into a fused, azygous(?) vessel. It then passed backward through the bifurcating halves of this vessel to the anterior part of the lateral fin-folds, where some of it went to the fin-folds through segmentally arranged branches somewhat like the metapterygial branch of *Heptanchus.* From this point posteriorly the remainder passed from the coracoids into their continuations, right and left lateral arteries, from which it was distributed segmentally to the fin-folds and to the sides of the trunk.

The subclavian artery, which in present-day vertebrates carries the blood supply to the pectoral area, arose in primitive elasmobranchs by the anastomosing of a segmental artery from the dorsal aorta and a branch from the coracoid artery.

The venuous blood from the fin-folds and from the sides of the body entered the longitudinal veins of the folds much as it now enters the lateral abdominal veins from the paired fins and from the sides of the body. Perhaps the fact that in elasmobranchs the lateral abdominal veins are among the first vessels to arise in the body of the embryo argues favorably for the primitiveness of these passageways. At any rate a considerable amount of blood on its way to the heart was collected in these longitudinal trunks. There is no reason, however, to think that in the areas which we now designate as pectoral and pelvic the blood from the fin-fold entered by means of large tributaries like the brachial and femoral veins, or from the body wall by the subscapular, but rather that it entered through veins which were small in caliber and segmentally arranged.

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EXPERIMENTAL AMOEBIASIS IN THE RABBIT

BY

MARGARET DORA THOMSON

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BY

MARGARET DORA THOMSON

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INTRODUCTORY AND HISTORICAL

Carnivores have been found to be apparently the most suitable animals for obtaining experimental infections of *Endamoeba dysenteriae.* The infection of a dog by Losch (1875) is the first case on record of the transmission of the amoeba from man to other mammals. Since then, amoebic dysentery has been produced in the dog by a number of investigators. By far the greatest amount of experimental work, however, has been performed with cats, which, like the dog, usually acquire a fatal dysentery.

Rodents have appeared to be rather refractory to infection by *E. dysenteriae.* Many of the attempts to infect members of this order of mammals have been without success. Kruse and Pasquale (1894) state that they inoculated two gerbils rectally with 5 cc. of a human dysenteric stool containing motile amoebae. No infection resulted.

Failures to produce infection in rats by feeding them cysts have been reported by Wenyon and O'Connor (1917), and Chatton (1917). The former used, however, only two rats, and the latter but one. Werner (1908) recorded an attempt to infect a few rats, but he does not give a definite account of the material or method used. Dale and Dobell (1917) tried without success to infect rats with a virulent strain of *E. dysenteriae* from cats. Recently, Wagener and Thomson (1924) were unable to produce an infection in any of sixteen amoeba-free rats by the rectal inoculation of actively motile amoebae from acute cases of experimental amoebiasis in kittens. No infection resulted from an operative injection of similar material directly into the caecum of four amoeba-free rats.

In 1917, Wenyon and O'Connor reported failure to infect a mouse by feeding it human faecal material containing cysts of *E. dysenteriae.*

A number of investigators, however, have recorded successful experimental infections in the rat and mouse. Lynch's report (1915) of spontaneous and experimental infection in rats has been questioned by Chatton (1917), Dobell (1919), and Kessel (1923). In Java, Brug (1919*a, b*) succeeded in infecting a wild rat, *Mus rattus,* by feeding it cysts of *E. dysenteriae* from man. He discovered spontaneous infection in two rats of this species. After a careful study of the amoebae found in a colony of white rats, *Rattus norvegicus,* Kessel (1923, 1924) carried on extensive experiments on the transmission of amoebic infections of man to rats by feeding them cysts. He succeeded in transferring to *amoeba-free* rats five species of human intestinal amoebae: *Endamoeba dysenteriae, Endamoeba coli, Councilmania lafleuri, Endolimax nana,* and *Iodamoeba bütschlii.* Of ten adult white mice which were fed cysts of *E. dysenteriae* and *Councilmania lafleuri,* one became infected with *E. dysenteriae* and another with *Councilmania lafleuri.* Unlike amoebiasis in cats and dogs, in which acute dysentery attends infection, the infection of *E. dysenteriae* in rats and mice appeared to be of a "chronic" type attended by the formation of cysts.

Unsuccessful attempts to infect guinea pigs have been recorded by Kartulis (1886), Hlava (1887), Kruse and Pasquale (1894), Werner (1908), and Wagener and Thomson (1924). In the first three cases, rectal inoculations of human dysenteric material were given to two, six, and three guinea pigs respectively. Werner does not give a definite account of his work. Among his experiments were attempts to produce an infection by direct injection of material into the liver. In the experiments of Wagener and Thomson, three methods were used. Twenty-three young guinea pigs were fed from four to seven times with mature cysts in human faeces, seven were given rectal inoculations of stools from kittens rich in actively motile amoebae, and two were injected directly by operation into the caecum with amoebae from experimentally infected cats.

There are two records, however, of successful attempts to infect this rodent. In 1914, Baetjer and Sellards gave a brief account of infection in three of four guinea pigs inoculated rectally with *E.*

dysenteriae. Two guinea pigs, which were inoculated with amoebae from human cases, died from bacterial peritonitis three days later. In both of them, *E. dysenteriae* was found in the bowel in an area of inflammation without ulceration. One of two guinea pigs, which were injected with bloody mucus from an infected kitten, died from a bacterial peritonitis in ten days. Typical endamoebae were found in an ulcer of the bowel. Unfortunately, the authors fail to give the exact location of the lesions in any of the animals nor do they give figures of the amoebae found therein. They state that none of the guinea pigs showed any symptoms of dysentery.

Chatton (1917, 1918*a, b*) succeeded in infecting guinea pigs, not only by rectal inoculation but also by feeding them cysts. Of three animals which ingested mature cysts, one became infected and died in twenty days. Scrapings from the caecal mucosa were inoculated rectally into two adult guinea pigs and a nine-months-old cat. Nine days later one of the guinea pigs died and amoebae were found in the caecum. The other animals did not become infected. An attempt to make a second transfer was unsuccessful. In both of the infected animals the modifications produced by the amoebae were confined to the caecum. One of the caeca was markedly thickened and congested. In both cases numerous white miliary abscesses were distributed over the surface. Sections showed a considerable thickening of the mucosa and that both the mucosa and submucosa were invaded by amoebae. Chatton (1917, 1918*a*) gives figures of the amoeba normal to the guinea pig, *Endamoeba cobayae*, and also of *Endamoeba dysenteriae* found in the caecum after experimental inoculation. Apparently there is no question concerning the nature of the infection that was produced.

In experimental work with rats, mice, and guinea pigs there is the possibility of confusion owing to the fact that there are intestinal amoebae normal to these rodents. Amoebae have been reported in rabbits by Walker (1908) and Brug (1919*b*). Walker describes two species, *Ameba enterica* sp. nov. and *Ameba fecalis* sp. nov., which he recovered in cultures from the caecum of the rabbit as well as from a number of other animals. In each species a contractile vacuole was a constant characteristic. Without question, the amoebae were free forms which had been ingested by the rabbits, possibly in the encysted stages. Brug found eight-nucleate cysts in the faeces of a single rabbit for a number of days, after which they disappeared. No motile forms were observed. Brug likened the cysts to those of *E. coli*, but since he was unable to prove their identity, he suggested a provisional new species, *Entamoeba cuniculi.* Whether or not Brug was dealing with an amoeba normal to the rabbit is questionable.

The rabbit has not been used extensively for experimental work with *E. dysenteriae.* Kartulis (1886) was unsuccessful in infecting a rabbit which he injected rectally with material from a human case. No details are given. Hlava (1887) gave to eight rabbits rectal inoculations of fresh stools from persons with acute amoebiasis. As in the case of the six guinea pigs he injected, none of the animals became infected, whereas two of seventeen dogs and four of six cats similarly treated gave positive results. In 1917, Dale and Dobell report unsuccessful attempts to transmit a virulent strain from kittens to rabbits by injection of the amoebae into the *small* intestine. The number of rabbits used is not mentioned. According to Chatton (1917) a rabbit which ingested 5 cc. of human faecal material containing numerous mature cysts and precystic amoebae failed to develop an infection. The person whose stool was used had been previously treated with emetine.

So far as is known, Huber (1909) is the only worker who has reported success in infecting rabbits. A number of times, eight rabbits were fed human stools containing cysts. Usually the animals passed normal firm stools, although a diarrhoea was noted occasionally. In no instance was blood or mucus observed. Although the rabbits ate readily they became very thin. One of them died in three weeks, another in five weeks, and the others were killed. Of the eight rabbits, four exhibited alterations in the intestine. As in the case of the guinea pig (Chatton, 1917, 1918*a, b*) the modifications were restricted to the caecum. Huber gives a description of the caecum of the first rabbit, which was modified more than any of the others. Both the outer and inner surfaces of the organ were of a spotted yellowish white color. Normally the wall of a rabbit's caecum is very thin and transparent, but in this case it had become thickened to a width of one to two millimeters. Numerous small ulcers were found on the inner surface, but nowhere were there deep or extensive ulcers. Microscopic study revealed enormous thickening of the submucosa, where amoebae were found in abundance. Apparently the alterations in the submucosa were due to a marked inflammatory infiltration. Many of the nuclei of the host tissue appeared to have lost their structure and were stained as dark homogeneous masses. Huber notes that the course of the disease in the rabbit differs considerably from that in the cat. Huber's account interested the writer in attempting a further investigation of the nature of the amoebic infection in the rabbit.

Grateful acknowledgment is made to Professor C. A. Kofoid and Dr. Olive Swezy for suggestions and criticism. and to Miss Inez Smith and Miss Anita Howard for valuable technical assistance.

MATERIALS AND METHODS

Repeated examinations of the stools of four rabbits three months old and one half-grown rabbit were made during two weeks prior to experimentation. The young rabbits were from a litter which had been raised in the animal rooms of the Department of Zoology of the University of California. All the rabbits were found to harbor coccidians and in the faeces of one were found cysts of *Giardia*. During the period of examination the animals appeared healthy, ate well, and passed only characteristic firm stools.

Later, examinations of twenty-eight rabbits from the same room were made. Of the twenty-eight rabbits, sixteen were approximately one month old, four were four months old, and the remaining eight were adults. In twenty-six animals, coccidians were found, in most cases upon the first examination. Six examinations failed to reveal coccidians in two of the adult rabbits. None of the animals appeared to suffer from the coccidial infection.

Faecal material containing mature cysts of *E. dysenteriae* for the feeding experiments was obtained from two persons with chronic amoebiasis. The diagnoses were made by Miss Inez Smith, technician for Professor Kofoid, and confirmed by the author before the material was fed to the rabbits. Permanent stained smears were made for later reference. During the routine examination of the two cases, a few cysts of *Endolimax nana* were found in an occasional specimen from case 1, and a light infection with *Endamoeba coli* was found in case 2.

For rectal inoculations dysenteric stools containing numerous actively motile amoebae were obtained from infected kittens (Wagener and Thomson, 1924). A hard rubber catheter was used to insert the material within the large intestine.

EXPERIMENT BY RECTAL INOCULATION OF A RABBIT

The half-grown rabbit was given an injection of 7 cc. of fresh bloody mucus from the stools of three infected kittens. The catheter was inserted 7.5 inches. Since the rabbit was apparently unaffected, a second inoculation was given twelve days later. At this time 10 cc. of similar material from three different kittens was inserted 12 inches within the colon. An examination of some of the remaining material after each injection showed that the amoebae were still actively motile. The rabbit remained normal, and the stools were unmodified and did not show the presence of amoebae.

Thus far nine rabbits have been inoculated rectally with dysenteric stools from human cases (Kartulis, 1886, and Hlava, 1887) and one with amoebae from infected cats. In no instance has this method proved successful in producing an infection. As with other rodents, there is considerable difficulty in inserting a tube rectally because of the presence of the hard balls of faeces and because of the danger of breaking the thin intestinal wall. Unlike the cat and dog, the rabbit has a long, much coiled large intestine, so that at best, material could be inserted for only a comparatively short distance. Since the caecum seems to be the region most susceptible to infection, rectal inoculation would appear to be an unsatisfactory method. The fact, however, that Baetjer and Sellards (1914), and Chatton (1917, 1918*a, b*) have been able by this method to infect the guinea pig, the digestive tract of which is similar to that of the rabbit, suggests that further attempts may prove successful.

FEEDING EXPERIMENTS ON RABBITS

Of the four three-months-old rabbits, three (rabbits A, B, and C) were fed human faeces (cases 1 and 2) containing mature cysts of *E. dysenteriae.* The fourth animal (rabbit D) was kept under normal conditions. Material from case 1 was given to rabbits B and C on February 20 and 21, and from case 2 to rabbits A, B, and C on February 28, 29, March 12, 13, and 14. The amount of material ingested each time varied from about 3 cc. to 8 cc. Although the animals continued for some time to eat about the usual amount of food, they became thin as compared with rabbit D.

On March 15, rabbit A did not eat and was noticeably ill. The following morning the animal was found dead. The autopsy findings were negative. Microscopical examination of fresh smears of the contents of the caecum and rectum showed the presence of coccidians, but not of amoebae. There were no noticeable changes in the liver or in the walls of the digestive tract.

By March 16, rabbit B had become markedly thin and was less active than normal animals. For about a week it remained in this condition and then gradually became stronger and gained in weight. By April 8, the animal had apparently recovered and appeared to be normal in every respect except that it was noticeably smaller than rabbit D, which had grown considerably in the six weeks. At no time during the period of experimentation were cysts or motile forms of *E. dysenteriae* found in the stools of either rabbit A or B.

On March 21, rabbit C was weak and ate very little. Prior to that date the animal had gradually become thinner, until there was a marked difference between it and rabbit D. During this time microscopical examinations of the faeces were made almost daily, but no amoebae were found. On the morning of March 22, the rabbit was found dying, but the writer was unable to work upon the animal until a few hours later. The caretaker for the laboratory animals reported that the rabbit had died about two hours before the autopsy was performed.

The liver was found to be apparently normal, and at first glance the digestive tract likewise appeared normal except for the congestion of the blood vessels, especially in the region of the caecum. Careful examination of the walls of the large intestine revealed no modifications in any region except the caecum. Here in the upper half was found a small area of yellowish white spots which appeared to be thickenings of the caecal wall. Upon opening the caecum a corresponding area of inflammation and ulceration was found on the inner surface. Microscopical examination of the caecal contents near this area was made at once and a number of amoebae were found. Most of them were rounded up but a few were sluggishly motile. In one case a distinct hyaline pseudopod was observed. Examinations of the contents of other parts of the caecum and of the remainder of the large intestine were negative. A later study of stained preparations of this material likewise proved negative. The tissues were fixed in hot Schaudinn's fluid. During an absence of the writer the preserved tissue was discarded. Fortunately a piece of the modified area of the caecum had been previously imbedded and sectioned, and was available for study.

The duration of the infection in rabbits cannot be compared with any exactness to duration in other rodents. Huber (1909) states that one of his infected rabbits died in three weeks, another in five weeks, and the ones remaining were killed. Unfortunately he does not give the length of time between the feedings and the time the rabbits were sacrificed. Moreover, Huber fed the rabbits cysts at intervals and he does not state whether the three and five weeks were calculated from the initial or from the final feeding. In the writer's case, the rabbits were likewise fed repeatedly. Rabbit C died 30 days after the initial feeding but the feedings were continued up to 8 days prior to the death of the animal. Thus it is impossible to determine definitely the duration of its infection.

The only guinea pig which has become infected by the ingestion of cysts (Chatton, 1917, 1918) died 20 days after a single feeding.

In neither infected rabbits nor guinea pigs have cysts been found, whereas in infected rats normal cysts occur frequently (Brug, 1919*a*, *b;* Kessel, 1923). Upon the last examination five months after the feeding experiments, Kessel found cysts of *E. dysenteriae* in the stools of infected animals. Of four of his infected rats that died, only one showed an intestinal ulcer. This was not examined microscopically to determine whether or not it was due to the amoebic infection. All these facts indicate that there is a marked difference between the nature of amoebiasis in the rat and in herbivores such as the guinea pig and the rabbit.

HISTOLOGICAL STUDY OF THE CAECAL WALL OF THE INFECTED RABBIT

Microscopical examination of sections of the ulcerated area of the caecum of rabbit C stained with iron haematoxylin showed that the caecal wall was heavily infected with amoebae. All the layers were found to be invaded. In most places the epithelium and a considerable part of the tunica propria were lacking. Apparently the amoebae were not the only agents in the destruction of the epithelium, for numerous bacteria were found in pockets beneath the intestinal glands and in other areas of the mucosa. Cysts of coccidians were found in the lumen of the caecum. *E. dysenteriae* occurred in the superficial débris of bacteria and fragments of cells, as well as in the underlying tissue of the mucosa which had not been destroyed. Not infrequently the amoebae were found invading the muscularis mucosa, and in a few places the muscular tissue had been entirely broken down. This is illustrated in figure 1 in which a rather deep ulcer can be seen on the lower right extending downward into a lymph nodule. Numerous amoebae are located at the base of this, and they also occur in abundance all through the remaining part of the glandular tissue extending below.

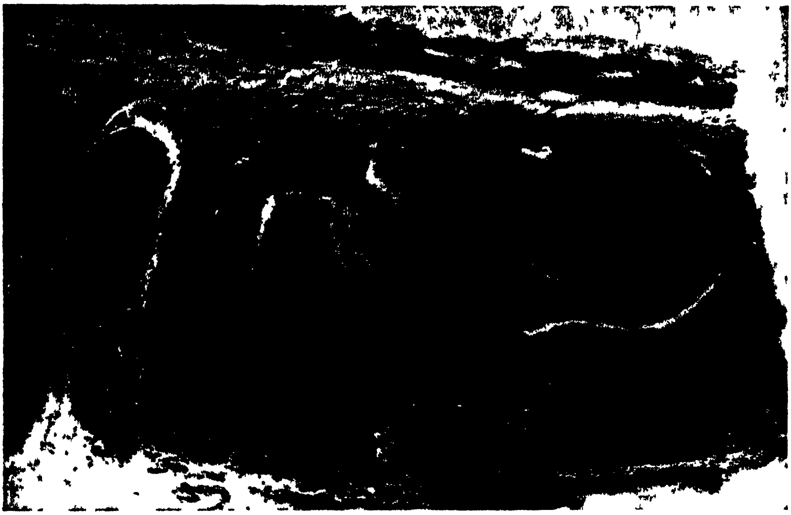

Fig. 1. Photomicrograph of section of the caecal wall of a rabbit experimentally infected with *Endamoeba dysenteriae*. The tissue was fixed in hot Schaudinn's fluid and stained with iron haematoxylin. Section of the wall in which all layers are invaded except longitudinal muscular layer and serosa. To lower left, ulcer extending into lymph nodule. X 55.

A number of adjacent lymph nodules were richly invaded. In some parts of a nodule the amoebae were massed together in such enourmous numbers that it was difficult to distinguish individual parasites. In one field, with a diameter of 0.163 mm., 55 nuclei were counted. In the network of lymphatic vessels in the outer part of the nodules, the amoebae were especially abundant.

The lower left side of figure 2 is an outer section of a lymph nodule, in which the amoebae can be seen situated in the clear spaces of the tissue. A considerable number of them are not in focus.

Many of the nuclei of the host cells were pyknotic and without definite form. Often the nuclei were stained as dark homogeneous

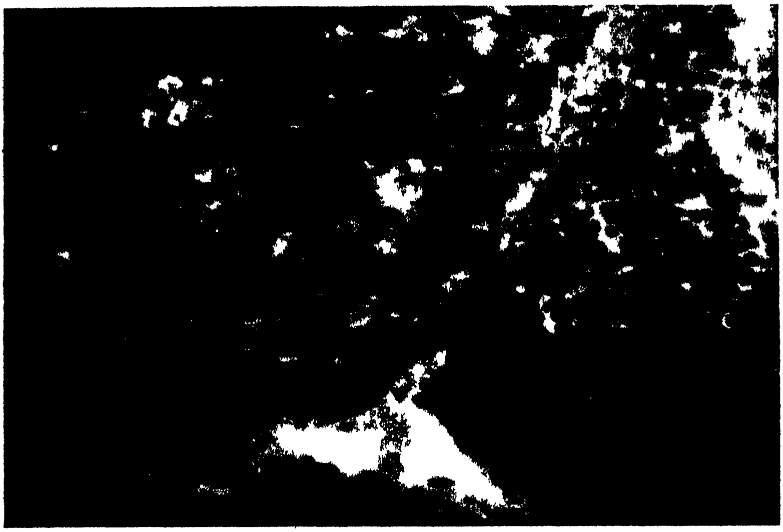

Fig. 2. Numerous *E. dysenteriae* located in the outer area of a lymph nodule of the caecum of an experimentally infected rabbit. × 600

masses so that no structures could be determined (fig 2). In many cases the cytoplasm could not be distinguished

In the lower part of the submucosa, practically all of the lymphatic vessels and spaces were found to be invaded by numerous amoebae Bacteria were also found occasionally. Figure 3 illustrates a group

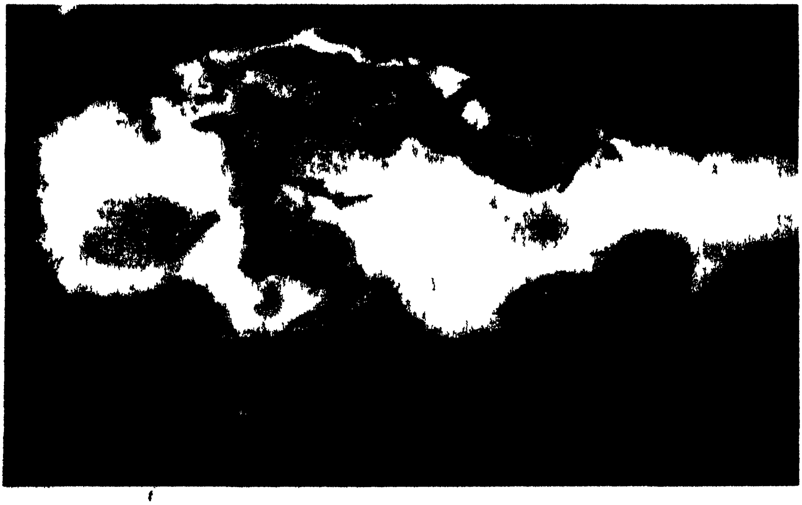

Fig. 3. A group of *E. dysenteriae* in a lymph vessel of the submucosa of the caecum of an experimentally infected rabbit. Note beaded peripheral chromatin and central karyosome in the visible nucleus. A few endothelial cells, stained black, in the lumen to right. × 1000.

of *E. dysenteriae* in one of the vessels. Unfortunately only one nucleus is in the plane of focus. It shows, however, the delicate peripheral layer of chromatin and the central karyosome characteristic of the species.

Recently, Boeck (1925) has recorded invasion of solitary lymph nodules and lymphatics in the colon of an infected cat.

In a number of capillaries and small veins of the submucosa amoebae were occasionally found, although never in abundance. Huber (1909) does not mention their occurrence in either the blood vessels or the lymphatics of his infected rabbits.

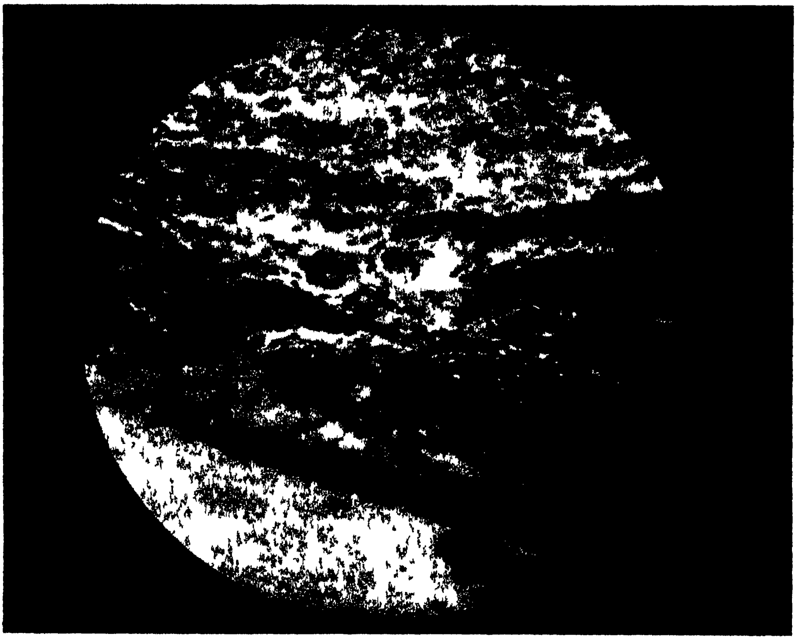

Fig. 4 Section through the wall of the caecum, showing the serosa (lower layer), muscularis, and a portion of the submucosa Note the destruction and invasion of the circular layer of the muscularis by amoebae. Numerous amoebae are present in the submucosa. × 335.

Even the circular muscular layer was invaded in a number of places. In figure 4 can be seen the destruction of the inner layer to the extent of over half its width. Figure 5 represents the central area of figure 4 taken at a higher magnification to show the nuclei of the amoebae occurring in the muscularis. The longitudinal muscular layer was not penetrated by direct invasion of the amoebae. Where it was pierced by lymphatic and blood vessels, however, amoebae were found located in the spaces about the walls of the vessels.

Very probably if the mesenteric lymph glands, aggregated at the base of the superior mesenteric artery, could have been examined, amoebae would have been found in abundance, since they occurred in the lymph vessels leaving the caecum.

Upon sectioning a number of swollen mesenteric glands from infected cats, Wenyon (1912) frequently found numerous amoebae. So far the author has been unable to find the organisms in sections of these glands from several cats infected with amoebiasis. The occurrence of *E. dysenteriae* in the lymph nodules and glands of infected animals is of interest in consideration of the possible relationship between amoebiasis and Hodgkin's disease in man, as pointed out by Kofoid, Boyers, and Swezy (1922*a, b*). It is to be regretted that Huber did not make a careful study of the lymph glands, since he had an abundance of material.

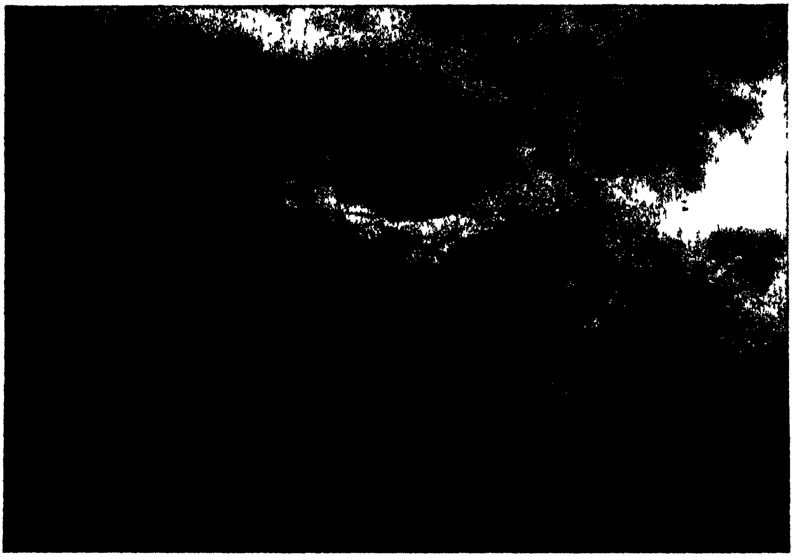

Fig. 5. The central area of figure 4 taken at a higher magnification to show the nuclei of the amoebae occurring in the muscularis. × 1080.

ATTEMPTS TO INFECT OTHER ANIMALS

Four half-grown dogs and one adult were fed human faeces containing mature cysts of *E. dysenteriae*. Prior to these feeding experiments Beatrice Howitt had succeeded in infecting the dogs with *Giardia* from human faeces. One of them received thirteen feedings of *E. dysenteriae* during a period of seven weeks, the second twelve feedings, the third and fourth two feedings each, and the fifth received but one feeding. In no case were there any dysenteric symptoms nor were amoebae found in the stools upon examination from five to twelve times.

Two young kittens (A and B) were fed mature cysts of *E. dysenteriae* occurring in the faeces of a rat which had been infected by Dr. J. F. Kessel. Prior to the feeding experiments, the stools of the cats were examined. No protozoa were found, but both cats were found to be infected with *Belascaris cati.*

Cat A was fed the rat faeces but once. The stools remained negative. Later it received seventeen feedings of cysts from human cases without result.

During a period of nearly two months cat B received nineteen feedings of cysts from the infected rat. Amoebae were never found in the stools of this cat, though fourteen examinations were made. A month later three feedings of human faeces containing cysts failed to produce an infection. When a few months later, in collaboration with Edna H. Wagener, a virulent strain of *E. dysenteriae* was obtained in cats from an acute infection in man, cat B was successfully infected after nine rectal inoculations with dysenteric stools from other cats.

SUMMARY

1. An unsuccessful attempt was made to infect a half-grown rabbit by rectal inoculation with motile amoebae in bloody mucus stools of infected cats.

2. Of three rabbits fed mature cysts of *E. dysenteriae* obtained from chronic human cases of amoebiasis one acquired an infection and died 30 days after the initial feeding.

3. No cysts were ever found in the stools during experimentation, nor at autopsy in the intestine of the infected rabbit.

4. The infection was localized in the caecum.

5. Microscopical examination of sections of the caecal wall showed an amoebic invasion of the mucosa, submucosa, and the circular muscular layer. Lymph nodes and lymph channels were heavily infected. Amoebae were also found in the lumen of the blood vessels.

6. Five dogs were fed human faeces containing mature cysts of *E. dysenteriae.* No infection resulted.

7. Unsuccessful attempts were made to produce an infection in two young kittens with cysts from an infected rat and later with cysts from chronic human cases. A few months afterward one of them was infected by rectal inoculation with dysenteric stools from cats with acute amoebiasis.

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ON STAUROJOENINA ASSIMILIS SP. NOV., AN INTESTINAL FLAGELLATE FROM THE TERMITE, KALOTERMES MINOR HAGEN

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INTRODUCTION

The study of the Protozoa living in termites is one of the most fruitful problems of protozoology. The work of Kofoid and Swezy (1919) on Termopsis and the recent studies on the relationship between termites and their intestinal Protozoa (Cleveland, 1923, 1924, 1925) have stimulated research by calling attention to the incompleteness of our knowledge of these animals. Scores of genera and hundreds of species remain to be described before any generalizations can be made, but when sufficient information is gathered the evolutionary series presented and the phylogenetic relationships which may be traced between host and parasite are certain to throw light on important biological problems. The relationship between host and termite has been very close indeed, since the Protozoa are very necessary to the life of the termite, as they digest cellulose in the intestine.

These studies will also aid greatly in determining the classification of termites. It is not always possible to distinguish termites by means of the workers, but an examination of the Protozoa serves readily to distinguish species from one another in the same locality, at least. Thus, for instance, *Kalotermes hubbardi* and *Kalotermes minor,* found in the same location, have slightly different intestinal faunas, as reference to the discussion below will show. The faunas are very constant as to species and relative numbers of individuals. Also, the associations of flagellates aid materially in allocating the termites to genera; as a check on the morphology of the insect, they throw light on the closeness of relationship; and they help in an understanding of geographical distribution.

Although there are at present twenty-four species of termites in the United States known to harbor Protozoa (all members of the Mastotermitidae, Kalotermitidae, and Rhinotermitidae harbor Protozoa), the present series is but the second recent attempt at a complete account of the Protozoa of one of them. *Termopsis angusticollis* Hagen was the subject of the thorough studies of Kofoid and Swezy in 1919. Leidy's accounts of the Protozoa of *Reticulitermes flavipes* Kollar were confused and incomplete, though a very valuable introduction to the field, and Porter's studies of the same Protozoa did not add very much to a knowledge of them. This study of *Staurojoenina assimilis* is the first of a series of papers on the flagellates of the termite *Kalotermes minor* Hagen, the others being species of *Devescovina, Oxymonas,* and a small tetramitid.

Kalotermes minor Hagen is one of the nine species of its genus which are found in the southern parts of the United States, and the first one of these to be studied. The only congener studied thus far is *Kalotermes flavicollis* Fabr. of southern Europe. Janicki published in 1915 a complete account of the flagellates of "*Calotermes castaneus* Burm. of Hawaii," but this was really *Neotermes connexus* Snyder, a native termite of the mountainous forests of the islands. *Kalotermes castaneus* Burm. is found in the West Indies and Florida.

Kalotermes minor is found in Lower California, California, and Arizona, where it occurs in dead wood on living trees in the cañons, generally in moist situations. The studies recorded in this series of papers were made on material collected from Mt. Diablo, California (November, 1924); Los Gatos, California (March, 1925); Gilroy, California (May, 1925); Prescott, Arizona; Sabino Cañon, Arizona (May, 1925), and Pasadena, California (June, 1925). The termites from Arizona were supplied by Professor S. F. Light, those from Pasadena by Dr. G. H. Ball.

From Ray and Phoenix, Arizona, Professor Light sent *Kalotermes hubbardi* Banks also, which proved very valuable for comparative studies.

In the intestine of *Kalotermes minor* there are four abundant flagellates. The largest of these is a species of *Staurojoenina*, which is present in all the individuals which harbor Protozoa. While not the most numerous, it attracts attention by its size and activities.

Kalotermes hubbardi also harbors *Staurojoenina*, which is, however, in a different association. The only other abundant flagellate is a form superficially resembling *Caduceia* Franca, 1918, but there is also a small tetramitid.

Grassi in 1917 described *Staurojoenina mirabilis* from *Epicalotermes aethiopicus* Silv., a termite of the northwest coast of Africa, thus establishing this unique genus. He devoted three pages and two plates to a detailed study of the morphology, but did not study it in living condition, nor had he any stages of the division process.

It is most interesting that the only representatives thus far known of this unique and highly specialized genus *Staurojoenina* have been found in one termite from the west coast of Africa and in two from Arizona and California. The flagellates are almost identical in the three, notwithstanding the wide geographical separation. *Stauro-*

joenina has not been found in *Kalotermes jouteli* Banks from Florida, *Kalotermes schwarzi* Banks from Florida, or *Kalotermes simplicicornis* Banks from Arizona.

This work was done in the laboratory of Dr. C. A. Kofoid under the James M. Goewey Fellowship at the University of California. I wish to express many thanks to Dr. Kofoid for invaluable assistance and criticism, and to Dr. Olive Swezy for help in preparation of the plates. Thanks are due also to Dr. S. F. Light for advice and assistance in obtaining termites, and for supplying data on *K. hubbardi* and *K. simplicicornis;* to Dr. L. R. Cleveland, who supplied slides of *K. jouteli* and *K. schwarzi;* and to Dr. T. E. Snyder.

MATERIAL AND TECHNIQUE

Staurojoenina assimilis is the largest and most delicate of the flagellates in the intestine of *Kalotermes minor,* where it has been universally found to be present, swimming actively among masses of *Devescovina, Oxymonas,* and a small tetramitid.

Studies of living material were made by diluting the contents of the intestine with 67 per cent Locke's solution and ringing with vaseline in order to prevent evaporation. Experiment had shown that in a 67 per cent dilution the animals lived longer, remained more active, and were less deformed than in other dilutions. The Protozoa as they flow out into the medium after the intestine has been torn suffer a shock, though *Devescovina* and the other flagellates do not seem to be much affected. Many, excepting those which remain close to the intestine, cease activity, but they soon recover and become very active. In higher concentrations changes of body form and finger-like projections from the surface appear, but in 67 per cent they continue more normal. They may be active for two or three hours, during which time some become deformed and die, but others live from twelve to twenty or more hours without much abnormality other than vacuolization of the cytoplasm.

The culture medium of Cleveland (1925*a*), was used to advantage, though it did not prove as successful as in his studies of *Trichonympha campanula.* A 0.2 per cent solution of NaCl proved too low a concentration and soon caused death of the organisms.

Preparations were fixed in Schaudinn's fluid with or without acetic, hot and cold; Flemming's fluid as modified by Gatenby, i.e., without acetic; and Bouin's picro-formol. They were then stained in iron-alum haematoxylin with and without counterstain of acid fuchsin or eosin; in Delafield's haematoxylin; in phosphotungstic acid haematoxylin. Methylen blue, Janus green, and neutral red were used as *intra vitam* dyes. It was sometimes helpful to show up the animals within a dark environment; for this purpose a suspension of India ink in 67 per cent Locke's solution was useful.

The best results in general observation were obtained with living material with no *intra vitam* dyes, and the best preparations were fixed in warm Schaudinn's with acetic and stained in iron-alum haematoxylin, with light counterstain of acid fuchsin or eosin.

Many slides were made on which no division stages were found; others had only a very few. By transferring the termites to filter paper and fixing them at intervals several slides were obtained, after about eighty hours, on which there were a great many stages of division. This experiment was repeated several times, and while often no division was found, frequently it was successful. A change in environmental and food conditions seems to be responsible for the onset of division in quantity which was rarely obtained in the ordinary way from wood-fed termites.

GENERAL MORPHOLOGY

Shape and Size

The form and size of the entire body of *Staurojoenina assimilis* is variable, but the anterior portion for a distance of about sixty microns from the tip is constant, due to the many elastic fibers supporting it. This portion (figs. A, B, D) is a broad-based, slightly convex cone, broadly rounded anteriorly, and bears the four bands of flagella. The diameter across the base is about sixty microns. The form of the body back of this varies, but is generally about that of figure B, that is, narrowly campanulate with rounded sides and broadly rounded at the posterior end. It may be more broadly campanulate (figs. A, 2; D, 4), so that the body, exclusive of the cone, almost forms a sphere; or the cytosome may be reduced so that it tapers inward instead of broadening towards the end (figs. A, 1; D, 3). In some cases (fig. A, 4), when very much elongated, it may contract slightly back of the base of the cone, continue with nearly parallel sides to a relatively great length, and be rounded or truncated at the end. Figure A, 5 shows the outline of a body when a portion of the cytoplasm is being cast off and it is much elongated.

This conical portion is divided into quadrants, so that there are four similar parts. The arrangement is expressed externally by the flagella, which are in four bands, and the four more or less prominent protoplasmic lobes between these bands.

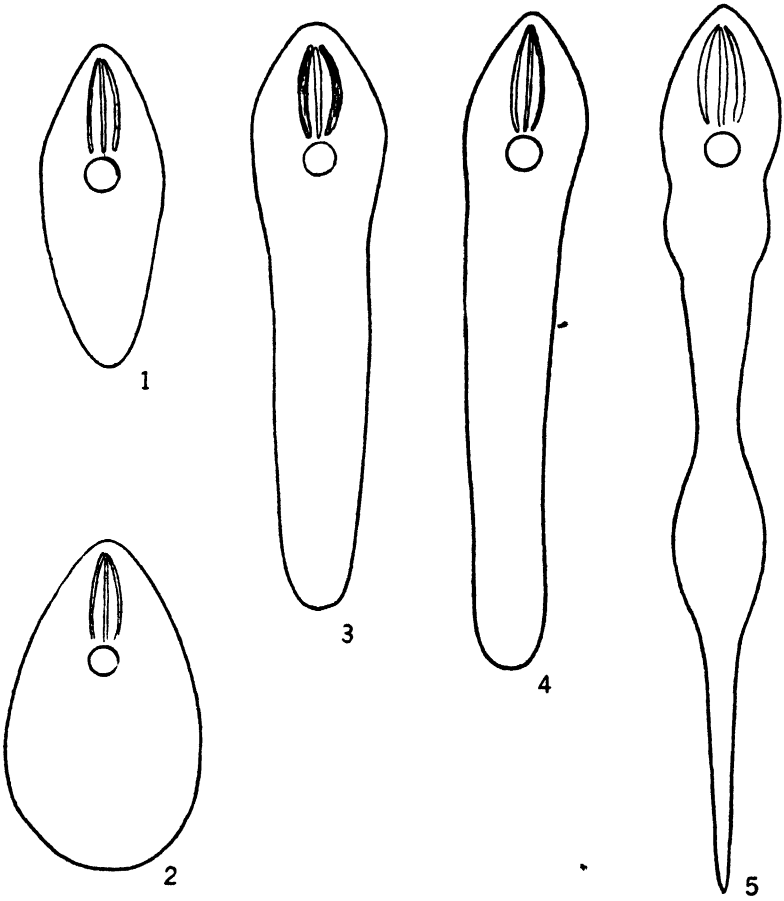

Fig. A. Outline figures of *Staurojoenina assimilis* to show form and size. 1, 2, the usual forms. 3, 4, very much elongated bodies, not very usual. 5, a much elongated body, part of the posterior protoplasm in process of sloughing off. × 333.

The length of fifty representative animals varied from 105 to 190 microns with the average at 142 microns. Lengths below 110 and above 160 microns are uncommon. A more constant measurement than the total length may be taken from the tip of the body to the posterior end of the nucleus, or better still from the anterior end of the neuromotor structures, the centroblepharoplast, to the posterior end of the nucleus (fig. B). The former distance ranges from 65 to 70 microns, with 70 microns by far the commonest measurement; the latter is almost constantly 60 microns, though a few vary from 57 to 65 microns.

NEUROMOTOR SYSTEM

The term neuromotor system is used for the organelles functioning in locomotion and conductivity, namely, the flagella, the basal plates, and associated structures, the centroblepharoplast, and the rhizoplasts connecting to the nucleus. Grassi (1917) has referred to these structures, in part, as the cytoendoskeleton.

The flagella are limited to the anterior portion of the body. They are arranged in four symmetrical bands (fig. B, *fl. bd.*) the bases of which are inserted in grooves of pyriform outline (pl. 1, fig. 1), and between these (pl. 1, fig. 5) there are more or less prominent protoplasmic lobes. Each of these bands has a greater breadth toward the middle than at the ends. The flagella at the anterior end are shortest, increasing in length until at the posterior end they are approximately equal to the body length. The bundle is composed of several hundred flagella. They stain readily with haematoxylin, and though they lose the stain more rapidly than the nucleus and body fibers, they retain it longer than flagella usually do. In preparations they are generally curved forward.

Each bundle of flagella is associated at the base with a shallow plate (pl. 2, fig. 10), convex outwardly and crescentic in cross-section (pl. 1, fig. 5). This plate is highly refractive, takes eosin and acid fuchsin, but it does not stain readily with haematoxylin. The four plates are arranged in a square (pl. 1, fig. 5), enlarging posteriorly, and approach closely at their edges. From these angles arise the four protoplasmic lobes. In the widest place, just posterior to the middle on the longitudinal axis, the plates are not separated even by as much as the diameter of the nucleus; the small basal granules of the flagella (pl. 2, fig. 10) are imbedded in their convex outer face.

When closely observed, especially when the organism is macerated, these plates are seen to be composed of, or to include, a number of longitudinal bands (pl. 2, figs. 9, 11, 12; fig. B, *periph. b.*), termed by Grassi peripheral lists (*listerelle periferiche*). Anteriorly these have a common origin from the four parts of the centroblepharoplast described below. The one on the right of each group is the largest, in the form of a long triangle which ends close to the region of the nucleus and continues as a long filament. The rest, about nine in all, are successively narrower and shorter, so that the left edge presents a curve and the shape of the whole is that of the base of the flagellar bundle. Each of these bands ends in a filament which passes out into the right side of the protoplasmic lobe which is to the left of the flagellar bundle corresponding to the plate. The filaments are successively shorter from the right, and so, while the right ones pass back of the nucleus, the others reach it. Apparently the filaments, and in part the peripheral bands, function as supporting structures.

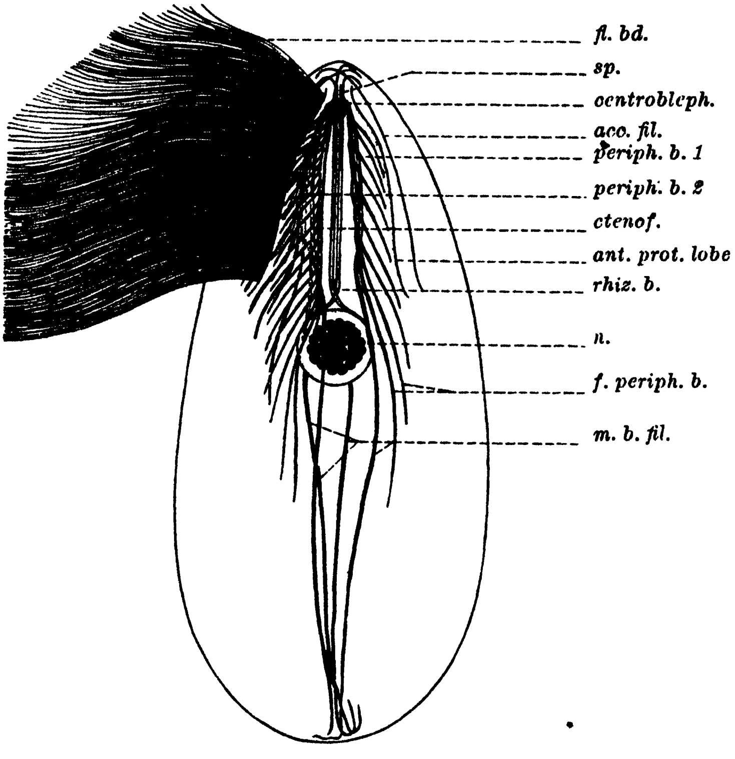

Fig. B. Diagram of *Staurojoenina assimilis*. Only one of the four bands of flagella is shown. The lateral edges of the upper protoplasmic lobe, the two series of supporting filaments in this lobe, two of the four flagellar plates, two of the four portions of the centroblepharoplast, and the rhizoplast bands are shown. The two centroblepharoplast bands are omitted. The posterior flagella are cut off.

Abbreviations: *acc. fil.*, accessory filaments; *ant. prot. lobe*, one of the four anterior protoplasmic lobes; *centrobleph.*, one of the four lobes of the centroblepharoplast; *ctenof.*, ctenofilaments; *f. periph. b.*, filamentous prolongations of the peripheral bands; *fl. bd.*, bundle of flagella; *m. b. fil.*, major body filaments; *n.*, nucleus; *periph. b. 1*, a series of peripheral bands; *periph. b. 2*, series of peripheral bands to which the flagella shown are connected, the portions of the flagella beyond the edge of the lobe being omitted for clearness' sake; *rhiz. b.*, rhizoplast bands; *sp.*, anterior spike-like prolongation of the centroblepharoplast lobe. × 650.

In macerations it may be plainly seen (pl. 2, figs. 11, 13, 14) that the flagella arise from these bands. Sometimes the flagella may be torn loose from them (pl. 2, fig. 8), the basal granules coming away also.

These peripheral bands sometimes take haematoxylin, but not readily. They stain with eosin and acid fuchsin, but may be seen very well in living material.

In the right side of each of the protoplasmic lobes (pl. 2, figs. 12, 14; fig. B, *ctenof.*) is a series of filaments which, from the general resemblance to a comb, may be called the ctenofilaments. These also have their origin from the lobes of the centroblepharoplast and number about twenty. Those at the left are the longest; thus the longest of these filaments is adjacent to the longest of the peripheral bands. The longer ones continue beyond the nucleus, but those at the anterior end are very short. They approach the periphery of the protoplasmic lobes, where they are recurved. Evidently they also may be elastic supporting structures, and together with the filaments of the peripheral bands constitute a double series of supporting strands in each protoplasmic lobe. These ctenofilaments do not come from a basal filament, but each seems to be connected independently to the point of origin, though all are gathered into a finer strand. The connecting portions are very fine and close together and are not always easily seen; therefore they often seem to emerge from an indefinite region, as Grassi has shown (Grassi, pl. 3, figs. 14, 17). These ctenofilaments stain black with haematoxylin, and take acid fuchsin and eosin readily. They are easily observable in living material.

The peripheral bands and ctenofilaments, as already stated, connect anteriorly to the four parts of the centroblepharoplast (fig. B, *centrobl.*). The term centroblepharoplast is used for the anterior portions of the structures designated by Grassi as radial bands (*listerelle radiali*), because the flagella and nucleus are ultimately connected to these and there seems to be no separate division center which can be termed a centrosome. Grassi's four radial bands are in the midline of the cytoendoskeleton, the rigid structure from which the flagella arise. Each is flattened and radially arranged (pl. 1, fig. 5), so that in section they form a cross. The anterior ends are more deeply stained and in life more highly refractive than the remainder, and each is in the form of an ax handle (pl. 2, fig. 9). Four spike-like processes (pl. 2, fig. 10, fig. B, *sp.*), one from each of these, project anteriorly for a short distance. Each of these four parts of the centroblepharoplast continues as a longitudinal band. Two of these bands, from opposite sides, extend back of the flagellar plates where they cross each other (pl. 1, fig. 7) and impinge upon the nuclear membrane. After touching this they turn outward and extend for a short distance along the membrane, but they are only attached to it, not fused with it, for the distal ends are often separated. These bands (fig. B, *rhiz. b.*) are homologous to rhizoplasts, but there is no evidence of a separate centrosome on the nuclear membrane. Grassi observed this contact with the membrane of the nucleus and figures it accurately (Grassi, pl. 3, figs. 2, 3, 8, 10, 11). He states, however, that through the contact of the four radial bands and the four major body filaments the nucleus is suspended, failing to note that these filaments are only adjacent to the nucleus, while the two rhizoplast bands are actually attached. The other two bands continue for a short distance as filaments, and may unite with the major body filaments described below.

There are usually four stout filaments (fig. B, *m. b. fil.;* pl. 1, figs. 1, 2, 4) extending back to the posterior end of the body deep in the endoplasm, and associated with each quadrant of the neuromotor apparatus. Since these do not develop completely until some time after division of the body they are not always all present. They stain deeply and are highly refractive in life, are elastic but seem not to be independently movable, as is the long filament in the endoplasm of *Pyrsonympha.* When some of the cytoplasm is discarded these filaments remain intact and become much curved for accommodation in the small space remaining. They pass close over, often in contact with, the nuclear membrane and separate into several strands just back of the flagellar plates. Some of these strands continue anteriorly as the longer left filaments of the ctenofilament series, others as the long right fibers of the peripheral band series, others as the two centroblepharoplast bands. Sometimes a major body filament is split throughout its length into two to four strands of nearly equal size. Thus these major body filaments are not single structures but comprise several united filaments.

The distance between the centroblepharoplast parts and the rounded tip of the body is of variable extent, but generally about ten microns. In this are the anterior portions of four accessory filaments (fig. B, *acc. fil.*) which seem to have no definite connections, lying in the center of the protoplasmic lobes between the ctenofilaments and peripheral bands and curving around anteriorly to add rigidity to the tip of the body. Posteriorly these accessory filaments extend to the region of the nucleus, and may be prolonged back of this as short filaments.

All these structures are best seen and studied in living animals held pressed between cover glass and slide in a suitable medium. They are then transparent and the flagella do not obscure the picture, as is the case in fixed and stained material.

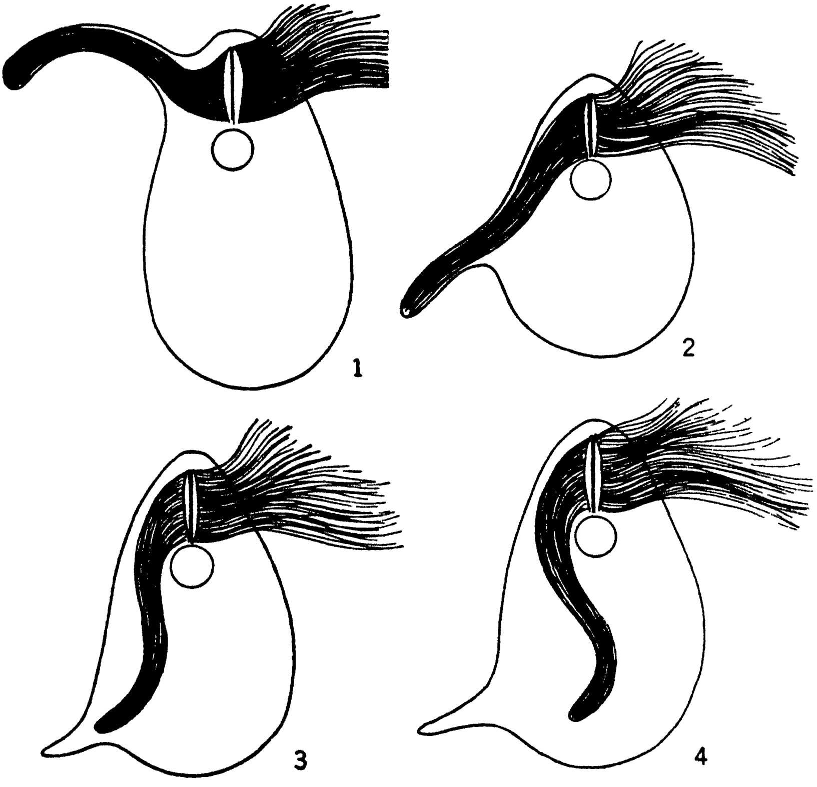

Fig. C. Diagrammatic figures of *Staurojoenina* sp. from *Kalotermes hubbardi* with an introverted bundle of flagella protruded as an arm beneath the pellicle.

The flagella are generally free, but sometimes (fig. C) the bundles are introverted, that is, they emerge from the plates as bands but are united distally into rounded bundles which lie entirely within the cytoplasm, turning back in the body. Grassi regarded these introverted bundles as characteristic of a second form of *Staurojoenina mirabilis*. From his account it would seem that this phenomenon was more common in his material than in *S. assimilis,* for among thousands of living and fixed animals only a few have been seen in which any bundles were introverted, and then there were only one or at most two in this condition, not all four. The intracytoplasmic bundles have been seen to break loose and become everted flagellar bands; at first, before eversion, the bundle is motionless; soon lashings begin, the end protrudes and becomes fimbriated; gradually it cuts through the body wall until the whole emerges and the bundle assumes the form of a band.

As shown in figure C, a bundle may push out the pellicle, so that a long protuberance is thrust out, covered by a thin layer of protoplasm. In figure C only two groups of flagella are shown, but there were two more free bands. The enclosed arm beat vigorously, eventually moved back under the pellicle as shown in the series of figures, then was withdrawn from its sheath and entirely enclosed in the endoplasm, where it moved with vigor and stirred up the cytoplasm.

No small introverted bundles were seen, so it cannot be said that they grow out under the pellicle and do not become free until of full length; but since it is hardly reasonable to suppose that free bundles are enclosed, it is most likely that such is the case. The flagella may, then, grow free from the first, as is usual; or may exceptionally grow internally until completed and then become extroverted. This fact, that flagella may for a time be intracytoplasmic, throws some light on the supposed origin of axostyles, which Kofoid and Swezy have regarded as intracytoplasmic flagella.

CYTOPLASMIC STRUCTURE

In preparations fixed in Schaudinn's fluid and stained in haematoxylin, with or without eosin counterstain, the endoplasm throughout the body appears finely alveolar (pl. 1, fig. 4; pl. 2, fig. 15), save for the neuromotor and skeletal differentiations. The portion of the cytoplasm posterior to the nucleus may be so filled with wood that the alveolar structure is only to be distinguished near the ectoplasm. Interspersed among these alveoles are numerous small granules, microsomes, which give the plasma a dense appearance. Under the periplast there is a narrow zone of hyaline ectoplasm, which appears narrower in fixed than in living material. In the outer part of the endoplasm just within this zone there are frequently numerous chromatic granules.

In living animals, taken from hosts which have been fed for a time on filter paper, the endoplasm appears quite clear and, under low power, homogeneous. Generally there are a number of moderate sized vacuoles in it, many of them empty, which are strikingly set off from the surrounding endoplasm. There may be little food material in the body, but often there are few to many cellulose particles. Careful observation shows many small alveolar spheres in the endoplasm, and a differentiated zone of ectoplasm which is also finely alveolar. Thus the fundamental structure is hyaloplasm in which are crowded alveolar spheres, larger alveoli or vacuoles, and abundant microsomes in the interalveolar regions.

CUTICULAR STRIATIONS

The surface of the entire body is uniformly and thickly beset (pl. 1, figs. 1, 6) with small rods of variable length. These are arranged in groups some of which are longitudinal, some transverse, some oblique to the axis of the body. This direction is as seen in preparations. They are visible in living material as refractive rods, uniform in thickness and distribution.

These are not bacteria, but are cuticular thickenings, for they are always present and are uniformly distributed. At the edges of the body it may be seen that they are prominences. As they are seen in living material, however, it is somewhat difficult to distinguish them from the bacteria which are very abundant on the surface of the body. There may be *Spirilla* in active motion, and large rod-like bacteria moving over the surface. These move over the striations and do not displace them, as they would were they bacteria. Also the rods never have any independent motion, as would be expected were they organisms adhering to the periplast.

NUCLEAR STRUCTURE

The nucleus is globular (pl. 2, fig. 8; pl. 3), surrounded by a well-defined membrane, and suspended by the two rhizoplast bands just back of the posterior ends of the flagellar bundle. The membrane of the nucleus is very delicate and is indistinct in many of the Mastigophora. In most termite flagellates it is more delicate than in *Staurojoenina,* in which it is certainly a definite and fairly thick structure. It may be observed in both living and stained flagellates and, in deformed nuclei, may be wrinkled. Likewise it is rigid enough to serve for attachment of the rhizoplast bands.

The internal structure of the nucleus is much like that of *Trichonympha campanula* and *Leidyopsis sphaerica.* The chromatin is collected (pl. 3, figs. 16–19) into an eccentric or central mass which has a diameter of half, or more, of that of the nucleus. In the interphase it is usually organized into large granules on a reticular framework. This mass may be so densely stained that no detailed structure can be made out. In stages of early organization (pl. 3, figs. 21–23) the granules may be smaller, more expanded, and eventually arranged into strands. This chromatic mass (karyosome) is surrounded by a layer of large, clear alveoles (pl. 3, figs. 16–18), between which there are minute granules and also processes from the chromatic mass. This alveolar zone is the same as that of *T. campanula,* in which "the alveoles are rounded outwardly and pressed close together on their inner faces. They are filled with a clear fluid which does not stain" (Kofoid and Swezy, 1919*a*, p. 58). Outside of this zone there is a zone of small alveoli, between which there are many minute granules. This zone may vary in extent and density, for when the inner chromatin mass is expanded the inner alveolar zone expands with it and crowds the outer zone. The outer zone corresponds to the granular area of the nucleus of *T. campanula.*

Thus the major portion of the nucleus is alveolar in structure, the chromatin collected toward the center. With the approach of the division process the chromatin is resolved into smaller granules and expands in strands which come to occupy the entire area.

This nuclear structure is more evident when eosin is used as a counterstain, but it is also visible with haematoxylin alone. The microsomes stain with haematoxylin quite lightly, but they take eosin well.

It is interesting to compare the two forms of vesicular nuclei, metazoan and protozoan, as found in these smears when there are many well-preserved nuclei from the intestinal epithelium of the termite. These are variable in size; many are of about the same size and shape as the nuclei of *Staurojoenina.* The termite nucleus is bounded by a more delicate membrane, and the interior is uniformly alveolar, or reticular, the alveoles or meshes intermediate in size between those of the two zones of the protozoan nucleus. The chromatin granules, the size of which varies from minute to that of the smaller chromatin granules of *Staurojoenina,* are distributed in the interspaces. They are especially abundant just under the membrane, but are also distributed generally throughout the area. At or near the center there are one or two chromatin-nucleoli, relatively large, rotund, and sometimes vacuolate.

The nucleus is faintly visible in flagellates just removed from the intestine, but after they have been kept in Locke's solution for a short time a gelation process sets in and the structures described above may be clearly seen. The animal may continue active for some hours after the nucleus becomes very distinct. The chromatin is gathered into an eccentric or central mass of small granules, or is expanded in strands (pl. 6, figs. 55, 56) throughout the nuclear area. No structure homologous to the "heterochromosome" of *Trichonympha campanula* and *Leidyopsis sphaerica* has been found.

OBSERVATIONS ON LIVING MATERIAL

Locomotor Activities

When first seen moving actively among the mass of flagellates and particles of wood removed from the intestine of the termite, *Staurojoenina* attracts attention at once by its agility and singular structure. The animal is in constant motion and the flagella continue beating until life is gone. The four bundles of flagella beat actively and independently. The animal is thus jerked from side to side but withal rowed forward, thrusting aside its lesser associates. The flagella thus aid in penetration of the seething mass and also serve as oars for locomotion. They are frequently moved forward at the base while still bent back distally, so that a shoulder is advanced; then they are thrust sidewise and backward, driving the animal forward and to one side. The opposite band thrusts it to the other side, and hence the jerky movements. Sometimes the animal remains in one place while the flagella move actively; at other times it moves steadily forward. In forward motion it does not revolve about its longitudinal axis as is the case with so many flagellates.

There is, of course, a great deal of débris in the preparations, consisting of the remains of the torn intestine, Malphigian tubules, and the intestinal contents. The animals when in the intestine swim through this last in a leisurely fashion, as they do outside after they have become accustomed to the unusual medium. They soon show a tendency to mass up against the intestinal fragments where they pack closely together and show little activity, save a more or less feeble movement of the flagella. An animal which is swimming about actively, ploughing its way through impediments, may eventually come into a corner in a mass of débris and persist in vain efforts to penetrate the mass, though it may be impossible to do so. After a time lobes of cytoplasm may be pushed out, possibly passively, between the débris and the cover glass. One animal was watched for more than an hour while it continued such vain efforts, becoming very suddenly active at times. At the end of the hour it still showed considerable agility.

An interesting observation showed how activity may continue in spite of almost complete separation from the nucleus, and how tough the protoplasm may become. A *Staurojoenina* was caught in the middle of the body by a Malphigian tubule which was pressed against the cover glass at one point. The animal was nearly cut in two by the pressure, and the posterior cytoplasm was separated from the anterior part save for two narrow lateral strands. The nucleus was in the posterior part, which was very much vacuolated. The flagella were in very active motion, and at times the whole body turned slightly around the protuberance, but in spite of the violent efforts made to escape, the connecting strands were not drawn out or disrupted. An hour and a half later it was in the same condition and quite as active, and after six and a half hours was still alive and moving, though feebly, the flagella then beating about once in two seconds.

DEGENERATIVE PHENOMENA

It will be of some value to record the phenomena of degeneration which begins soon after removal from the intestine and proceeds until after twenty-four hours, when, in the medium used, most of the animals are dead. In the unaccustomed medium, while *Staurojoenina* lives for a time, it evidently suffers some osmotic changes, which are fewest in the optimum concentration. The body becomes irregular and abnormal in form and narrow, finger-like processes project from parts of the body surface. The regions between the flagellar bundles expand into much more prominent lobes than is normal. The finger-like processes are commonest on these lobes, especially their anterior portions, though they may also occur elsewhere on the body. These projections are rather rigid. They do not move, save against the resistance of the environing fluid, and change form little if at all.

Soon after removal from the intestine the bodies round up and these finger-like processes appear more or less abundantly. Vesicular bulges, which may cause a good deal of tuberosity, are produced by protruding vacuoles and are therefore clear, while these projections are granular, that is, protoplasmic. The flagella disintegrate from the distal end, resolving into granulations. The tips are degenerated in some that are still alive, and in dead organisms they are completely destroyed. The periplast seems to be rigid, for it wrinkles up on deformed bodies. Before death the protoplasm becomes greatly vacuolated; some of the vacuoles are small, others large and they are set off sharply from the granular. protoplasm. Particles of wood may be scattered between and in these vacuoles. When degeneration has progressed so far that the body is but a vacuolate sphere, the neuromotor and skeletal structures are still intact with suspended nucleus and cytoplasmic filaments. The nuclear wall is still intact, but the contents may be in a state of dissolution. The neuromotor apparatus, excepting the flagella, is very resistant, and persists when the body is ruptured and the cytoplasm dissolved.

Sometimes, under adverse conditions, the body constricts back of the nucleus until the wood-containing posterior portion, having become globular, is separated from the anterior part. The wall closes over until this anterior portion is complete and rounded off posteriorly. The four long cytoplasmic fibers, which ordinarily reach to the posterior end of the body, are curved back so that they may be accommodated in the reduced cytosome. This isolated neuromotor system, having discarded all but a minimum of cytoplasm, may continue active for a time.

FOOD AND FOOD-TAKING

It has been difficult to determine the method by which the flagellates of termites ingest the woody particles which are generally abundant in the endoplasm. Doubtless all these hypermastigotes feed in a similar way, conditioned by the facts that there is no cytostome, that much differentiation of the anterior part of the body is present, and that the posterior part of the body is mobile and the pellicle there relatively thin. It has generally been stated that the food particles are enclosed by the body protoplasm. Food ingestion in *Leidyopsis* and *Trichonympha campanula* has recently been studied by Swezy (1923) and Cleveland (1925). The former author described and figured the method of intake of large particles of wood by *Leidyopsis* and *Trichonympha.* These, coming into contact with the body, may be caught by the sticky, mobile ectoplasm of the region; clear pseudopodia are then thrust out along the wood and it is eventually engulfed, the body often distending to accommodate it. *Trichonympha, Leidyopsis, Staurojoenina,* and others often contain such large pieces of wood, but more commonly the endoplasm is filled with much smaller pieces. Cleveland (1925) observed that in the ingestion of these small particles a cup-like cavity or "temporary cytosome" is formed at the posterior end of the body, drawing in the wood particles which adhere to the end. The cytoplasm then flows backward and narrows the cavity until it is completely closed, and the resulting vacuole is bordered by what was the pellicle of the body. Such cup-shaped cavities in the posterior end are often seen in *Trichonympha campanula.*

Figure D shows the endoplasmic inclusions of *Staurojoenina* under different conditions. When the flagellate is removed from a termite which has been feeding on wood under favorable conditions, it is generally crowded with small bits of wood as in figure D, 1. Less frequently there are large pieces (fig. D, 2) and sometimes these are so large that the body is much distended, even stretched out as is *Leidyopsis* in Swezy's (1923) figures 10 and 11. Vacuole walls may be seen about most of these particles and are probably always present, though often they are so close to the fragment that the latter seems to lie in direct contact with the endoplasm. In figure D, 4 there are numerous large vacuoles, each of which encloses several fragments of cellulose. This termite has been fed for several days on filter paper. Under such conditions the cytoplasmic inclusions are quite different from those usually seen. The body is generally less crowded and may often, after prolonged diet on filter paper, be clear and free of cellulose. Large vacuoles containing small bits of cellulose are common. Often there are many vacuoles as shown in figure D, 3, 3*a*, and each of the larger of these, besides containing some food particles, contains many *Spirilla,* which are usually in active motion. Less commonly vacuoles contain small flagellates, the tetramitid common in *K. minor,* which are often very active also. Such inclusions are not usual under normal food conditions, so that their presence is evidently connected with the disturbance associated with cellulose feeding. It is not certain that they are used as food, although present in vacuoles, for generally they are active, but their presence in such large numbers when particles of wood are scarce raises the question whether they are or not.

As already stated bits of wood may adhere to the flagella and the surface of the body, especially at the posterior end. Pseudopodia such as those shown by Swezy (1923) in *Leidyopsis* are often thrust out in contact with these fragments. Although no observations of actual food intake have been made, there seems little doubt that it occurs in this way. No cup-like invaginations such as Cleveland described in *Trichonympha* were observed, so probably the small

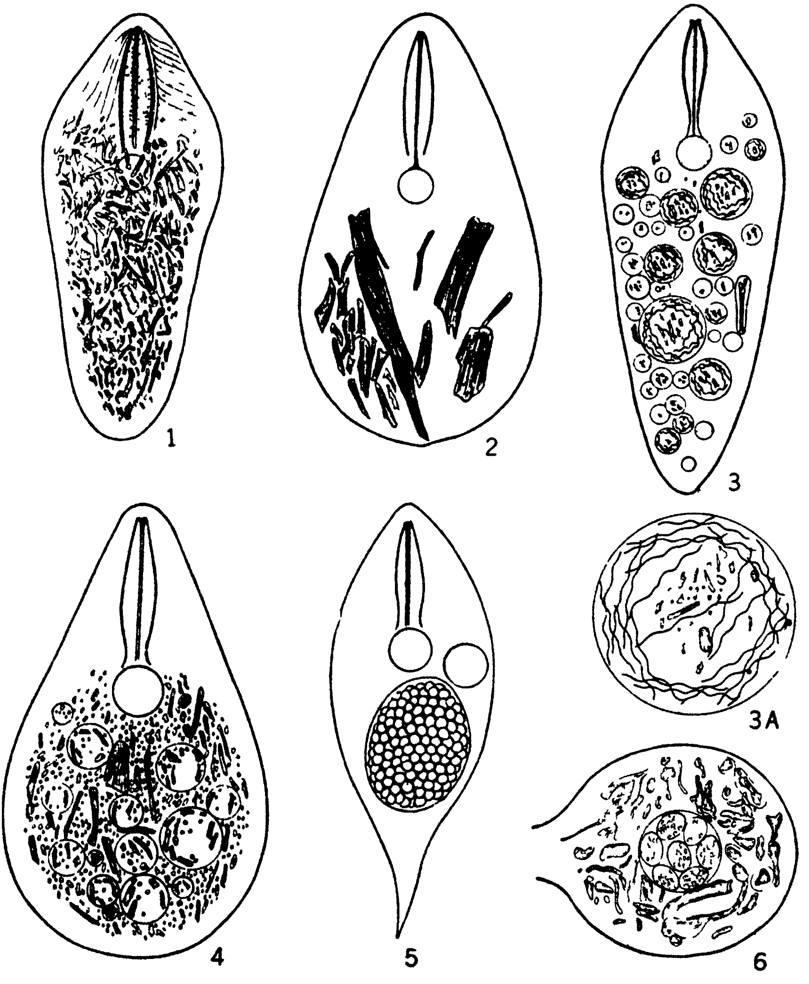

Fig. D. Cytoplasmic inclusions of *Staurojoenina assimilis*. The outlines show various body forms. 1, body filled with small particles of wood, the most usual appearance in those normally fed. 2, large particles of wood in cytoplasm. 3, vacuoles containing active Spirilla, in flagellate from termite fed for several days on filter paper. 3*a*, enlargement of a vacuole containing active Spirilla. 4, large vacuoles containing particles of cellulose, in a flagellate from a termite fed several days on filter paper. 5, a large granular sphere in the body, probably a parasite. 6, posterior portion of body containing particles of wood and a large vacuole in which are some small active flagellates.

particles are taken in more gradually. One would expect to find large vacuoles surrounding many fragments if ingestion occurred in this way, but such large vacuoles are uncommon. Ingestion has been observed elsewhere on the body surface, even anteriorly in the protoplasmic lobes.

In animals fed on filter paper several singular phenomena have occurred in the anterior region of the body. The protoplasm there seems very mobile, and frequently long processes are protruded. Generally these are not independently movable, but often a stout process capable of movement appears. Due to undulations of some structures these processes appear to be in rapid rotation. Probably this effect was produced by active *Spirillum* caught in the "pseudopodium." These processes were at times drawn out to a relatively great length, with undulating *Spirillum* at the end giving the appearance of a boring augur. This could be retracted again, bent about actively, even turned back over the body. From a region near the base of this, or more distally, a second apparently whirling process could be thrust out and again entirely retracted. A *Spirillum* was seen protruding from the end of a similar process. A *Spirillum* which is within the "square" may migrate into the posterior endoplasm, though slowly. They are often seen in the protoplasmic lobes and near the tip of the body. Farther back they sometimes penetrate the body wall and become enclosed in a vacuole in the endoplasm. There is not a little evidence, therefore, that bacteria at least may be captured by pseudopodial activity at the anterior end of the body, or perhaps captured by the sticky ectoplasm which they draw out into "pseudopodia," as well as taken in posteriorly.

Long processes are often thrust out from the protoplasmic lobes. Sometimes these are certainly due to the abnormal condition produced by the non-isotonic medium, but some are apparently normal. A long piece of wood is sometimes held or partly surrounded at the end by such processes, and in several instances the wood was already partly within the body of another animal. In one such case, illustrated in figure E, two animals when first seen were attached to the same piece of wood; one by a process projecting from the anterior portion of a lobe and actually wrapped around one end of the wood, the other by the posterior end of the body which had engulfed the other end. These two were turning around one another (fig. E, 1). After a time (fig. E, 2) they turned in opposite directions and began swimming vigorously, the arm of one remaining attached to the wood and drawing out very little, a demonstration of the toughness of the protoplasm. This pulling resulted in drawing the wood back to the end, drawing out the body of A, which held it very tenaciously, but finally, very slowly, let it go. In figure E, 3 the wood had been removed, but still adhered to the protoplasm. Flagellate B then turned around, after lodging the wood against an obstruction, and the arm moved back toward the posterior end (fig. E, 5), still remaining attached to the fragment. Eventually B brought its operculum against the end of A and dislodged A from the fragment. By this time (fig. E, 6) the arm had become a tail, having shifted to the end of the body. A swam away, B swam forward, but the wood, held against the obstacle (fig. E, 7), hindered progress. The flagellate did not change its direction but kept pulling until the wood was drawn around and unimpeded progress was again possible. Soon the fragment lodged against

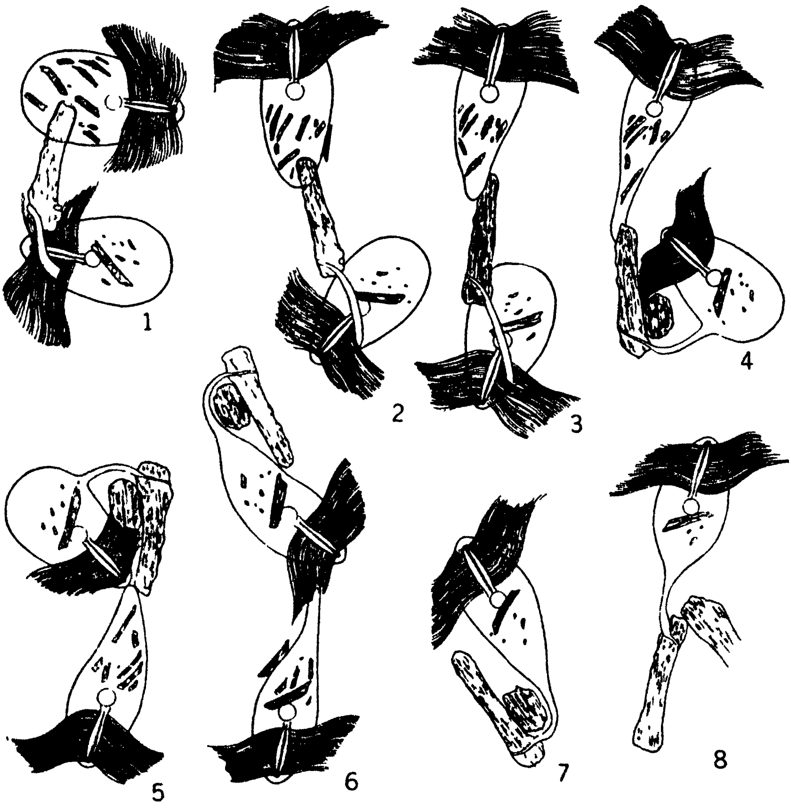

Fig. E. *Staurojoenina* sp. from *Kalotermes hubbardi*. A series of outline drawings showing how one flagellate, with an anterior arm attached to a piece of wood which is partly within the body of another, pulled it away from that other. See text, pp. 44–46, for explanation. × 190.

some débris, and for an hour the flagellate continued vain efforts to pull away. During this time the "tail" did not pull out at all. However, after some time the flagellate broke loose and swam away. On several similar occasions a flagellate, holding by its anterior lobes to a piece of wood, was not so successful in getting it away from another.

It is difficult to explain this behavior. It seems to be an actual appropriation of a piece of wood belonging to one flagellate by another, or else assistance given by one in removing the wood from another. In the cases observed the wood was rejected by the second possessor, and it does not seem likely that it would be ingested again. There could hardly be an intentional aid or taking away, such purposeful behavior being out of the realm of such simple animals. Probably the wood merely happened to adhere to the anterior lobe, which was drawn out, but then it is difficult to explain why the arm ceased extending at a certain length and became very tough. The observation shows how adhesive the protoplasm is at times and what strains it is capable of undergoing without rupture, and also how such a protuberance may be shifted in position.

FISSION IN LIVING MATERIAL

Longitudinal fission of the body was sometimes observed in living animals. This takes place as is usual in flagellates, the groove beginning at the anterior end (pl. 7, fig. 61) and extending back until the flagellated ends are opposite to one another (pl. 6, fig. 58). They then pull apart, each of the products of fission taking two of the four flagellar bundles. One pair observed (pl. 6, fig. 57), where division had not been normal, were connected by a strand 350 microns long. This strand became wrapped around débris and remained intact in spite of considerable pulling. It was evidently very tough. The pair of flagellates was watched for an hour, during which time they remained connected, the strand pulling out to a length of 450 microns.

BINARY FISSION

The mitotic process is initiated by nuclear changes. The mass of chromatin, which is very compact in the resting stage, increases in size and becomes looser. The granules may be irregular in shape, variable in size and unevenly distributed in the reticulum. Later, granules (pl. 3, fig. 20) are more uniformly distributed, are rotund and equal, and number not less than 70–80. The inner zone of large alveoles expands and crowds the outer finely alveolar zone until this is almost suppressed save for granulations under the nuclear membrane. The chromatin then organizes into a continuous, relatively small strand which is much coiled throughout the entire nucleus. It is not absolutely certain that this strand is continuous as one, but there are cross-connections which connect it all together. The chromomeres are strung in a row on the supporting fiber, like a loose string of beads, more or less separated. The strand, if ever single, soon breaks into segments which shorten as the chromomeres are drawn closer together until the individual outlines are not so readily discernible, though still irregular. As these strands (pl. 3, figs. 24–26), of which there are twenty-four, become more massive they clump toward the center of the nucleus, leaving a clear peripheral zone.

During these changes the paradesmose forms out of the cytoplasm. In most flagellates the paradesmose is a deeply staining strand or bar against the nuclear membrane, connecting the centrosomes. There are no granules which may be termed centrosomes in *Staurojoenina,* but the rhizoplast bands which impinge on the nuclear membrane are closely related to the organizing paradesmose. The development of this structure is not exactly synchronous with the nuclear changes. Sometimes it appears while the chromatin is in loose tangled threads, but at other times it does not form until later. At first (pl. 3, fig. 24) there is a granular density of the cytoplasm above the nuclear membrane between the two slightly separated bands. Later (pl. 3, fig. 30) a few fibers appear in this region between the bands, which spread apart. These fibers are relatively stout, and stain with haematoxylin. Only the lower ones are against the nuclear membrane; the rest are free from it but are connected to the rhizoplast bands more anteriorly.

The shortened, thickened spireme threads assume a more open arrangement and split lengthwise (pl. 3, fig. 28; fig. F, 1). The chromomeres may still be distinguished. The apposed chromomeres of a pair are similar (fig. F, 2) which is evidence that they are equally split. In different chromosomes nine to thirteen granules which differ from one another in shape and size have been counted. The arrangement of the chromosomes is at first irregular, but changes so that these are parallel, directed toward the region of the nucleus under the paradesmose, and are co-extensive (pl. 3, fig. 29). These bivalent chromosomes (pl. 3, figs. 29–31; pl. 4, fig. 33) shorten and thicken until the individual chromomeres are not distinguishable. The members of a pair approach until the split disappears (pl. 4, fig. 36) and there are twenty-four short rods formed by fusion of the bivalent elements.

Meanwhile development of the paradesmose continues. Separation of the rhizoplast bands progresses anteriorly until the entire centroblepharoplast separates into two parts (pl. 7, fig. 59), the flagellar

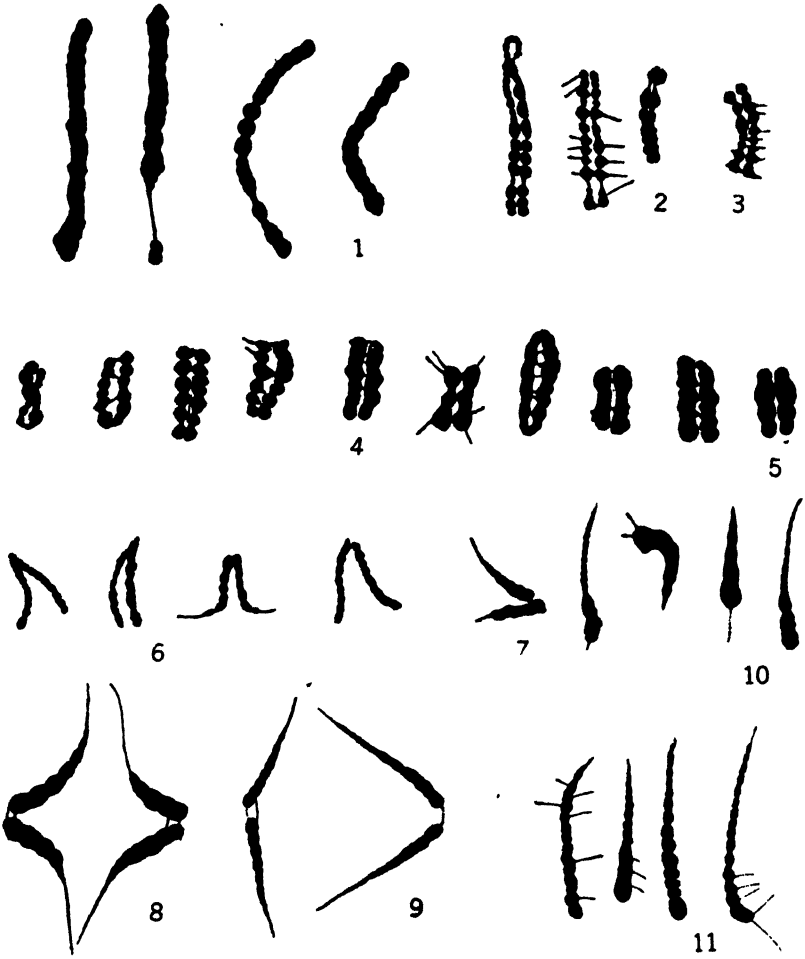

Fig. F. Chromosomes of *Staurojoenina assimilis*, taken from figures on the plates. 1, four chromosomes (fig. 28). 2, two chromosomes (fig. 30). 3, one chromosome (fig. 32). 4, four chromosomes (fig. 31). 5, five chromosomes (fig. 34). 6, two chromosomes (fig. 38). 7, three chromosomes (fig. 37). 8, anaphase chromosomes. 9, two chromosomes (fig. 40). 10, four chromosomes (fig. 41). 11, four chromosomes (fig. 50). × 3000.

Figure 1 shows splitting of the chromosomes, figures 2–5 paired chromosomes, figures 6–9 the method of separation, figures 10 and 11 separated chromosomes of anaphase.

plates and associated structures separate into pairs also. The paradesmose (pl. 4, fig. 42) becomes a thick bundle of coarse, chromatic fibers shaped in longitudinal section like a plano-concave or even a double-concave lens, squared at the ends, along which the rhizoplast bands course, round in cross-section (pl. 5, fig. 45). Under the paradesmose the nuclear membrane is pushed in so that the paradesmose comes to lie in a depression (pl. 4, fig. 42; pl. 5, figs. 44, 45), as can readily be seen when it is on end. The chromosomes are directed with one end toward the spindle, parallel to one another or converging slightly. From the nuclead corners of the paradesmose many astral rays (pl. 4, figs. 41, 42) radiate into the cytoplasm. Some of these are about as long as the nucleus.

With extension of the paradesmose and elongation of the nucleus the chromosomes separate (pl. 4, figs. 37, 38; fig. F, 6, 7) along the line of the previous split, beginning at one end and pulling out into V-forms. They are then approximately parallel to the paradesmose, and the two ends approach the poles. Finally (fig. F, 8, 9) the arms of the V's pull apart at the apices and the chromosomes thus separate into two groups (pl. 4, figs. 40, 41), each converging toward a pole.

The nuclear membrane has remained intact. The nucleus is now elongate oval (pl. 5, fig. 44) and contains two groups of chromosomes. A large, fibrillar paradesmose stretches from pole to pole and lies in a depression of the nucleus. From the poles astral rays radiate into the cytoplasm. A rhizoplast band runs past each end of the paradesmose and to it the fibers are connected. The neuromotor structures have separated into two parts which lie parallel at some distance from each other. Plasmotomy has just begun with an anterior depression.

After the nucleus has drawn out to more than double its original diameter it constricts in the middle (pl. 5, fig. 47) while the chromosomes are in dense masses at the poles. There is an aggregation of granules under the nuclear membrane, and a concentration (pl. 5, fig. 48) of these at the waist of the nucleus. The constriction deepens pl. 5, fig. 48) to complete separation (pl. 6, fig. 49), division taking place between the aggregated granules. The nuclei when separated again become spherical.

The paradesmose does not become greatly elongated, as in some other flagellates, but separates in the middle (pl. 6, fig. 50) while still entirely above the nucleus, a part passing to each daughter individual. One end remains attached to the rhizoplast band (pl. 6, fig. 51), the fibers becoming spread out proximally and drawn together at the distal end, where they extend into a filament. The paradesmose eventually disappears, but disintegration proceeds slowly. The structure is evidently very resistant gel, and does not revert to the sol condition for some time.

Fission of the body begins in the anaphase and is completed before the nuclei are reorganized, so that in recently divided animals the chromosomes are still converging threads and the paradesmose persists. Since growth of the two new groups of flagella and associated fibers does not begin until after binary fission, each of the two new individuals has only two groups of flagella, two protoplasmic lobes, and two of the long chromatic major body filaments.

The chromatin, which in the early telophase (pl. 6, fig. 51) is still organized in converging strands, is later loosened up and resolved into fine, granular, convoluted threads which fill the nucleus. These strands break up, the chromomeres aggregate, and finally the mass of large granules appears separated by the two series of alveoles from the membrane, which is characteristic of the resting stage.

Only two of the bands from the centroblepharoplast have been followed through this process, namely, those which are attached to the nucleus and separate to the daughter nuclei, dividing later on to form two suspensory bands for each. The other bands end freely in the cytoplasm and, in late prophase and anaphase stages, seem to be associated (pl. 6, fig. 49) with the outer paradesmose fibers. With division of the body these separate. They subsequently swell and become of about the same consistency and extent as the flagellar plates. A second band appears opposite to the first. These bands subsequently become the basal plates of the two new flagellar bundles (pl. 7, fig. 62) which begin to grow soon after division is completed. At first (pl. 7, fig. 62) the flagella are very fine and short and do not cover as large an area as the full grown flagellar bundles, nor are the grooves from which they emerge coextensive with the original grooves. They grow (pl. 7, fig. 63) until the new bundles are equal to the original ones.

While development of the ctenofilaments and major body filaments could not be followed, it is most likely that they grow back from the centroblepharoplast, according to the usual method of flagellate organelles. The two new filaments are at first fine and short, and later increase in size. They are not completed until some time after reorganization of nucleus and flagellar bundles. Sometimes new ctenofilaments, finer than when full grown, are to be seen beside fully developed flagellar plates.

Many stages of this process could be followed in living material, which, however, had been under the influence of the environing fluid for some time. When the animals are first released from the intestine the nucleus is optically homogeneous, surrounded by a thick membrane. Soon large granules appear, forming a mass at the center as in the resting fixed nucleus. The increasing visibility is probably due to the coagulation precipitated by the unusual conditions, but it is likely that these structures are already present as such. Spireme stages (pl. 6, figs. 55, 56) are not uncommon. The appearance of the threads is similar in fixed and living material, so that there is evidently no change caused by fixation and staining. A later stage (pl. 6, fig. 54) where chromosomes were formed, possibly by splitting, and connected together by highly refractive threads, may have been somewhat abnormal. The fibrous paradesmose and rhizoplast bands (pl. 6, fig. 54) can also be easily seen. When seen on end the paradesmose appears sunken deeply in the kidney-formed nucleus. the membrane of which remains stout and intact. In telophases the knobby chromosomes converging to the poles (pl. 5, fig. 53) are very distinct; some chromosomes are connected to the antipolar wall of the nucleus by stout threads. In these telophases the paradesmose may also be observed attached above the nucleus to the rhizoplast band.

MULTIPLE FISSION

What seemed to be a case of multiple fission was observed only once (fig. G) in a living animal. A large plasma mass, filled with small particles of wood, was divided up peripherally into projecting lobes each furnished with four active bundles of flagella. The flagella were vigorously pulling in different directions, the whole plasma mass moved slowly. There were eight individuals. each possessed of a nucleus and four flagellar bands, some of them more free from the general mass than others. During the hour of observation they separated some. but soon after, under the adverse conditions. the whole animal, save for a very feeble movement of the flagella. was dead.

This might be a case of multiple fission, or might be fusion, but the latter seems unlikely. While in ordinary binary fission there are only two flagellar bundles, the others not having grown, in this case the new ones had been completed in each individual.

Multiple fission among other hypermastigotes has been recorded in *Lophomonas* only. It is a rare phenomenon in the hypermastigotes of termites, but is not uncommon in certain polymastigotes, as *Trichomitus termitidis* and *Streblomastix strix* (Kofoid and Swezy, 1919).

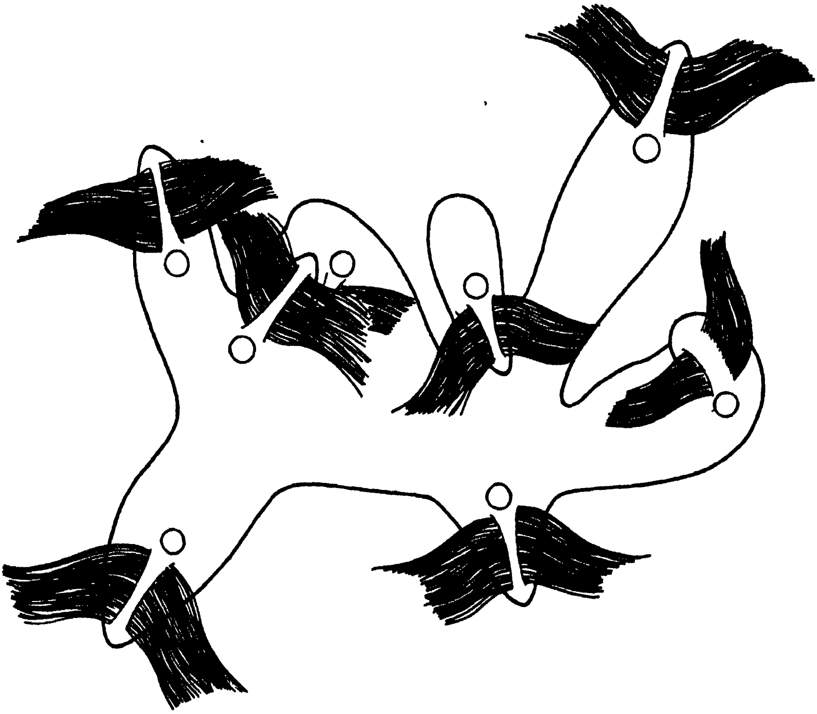

Fig. G. A probable case of multiple fission in *Staurojoenina assimilis*.

BINUCLEATE FORMS

Several binucleate but otherwise normal and mature animals were found on slides. In such cases one nucleus was attached normally by the rhizoplast bands, and the other lay free in the anterior part of the body. This phenomenon was due, probably, to abnormal division, the nucleus having divided without separation of the other elements.

SYSTEMATIC REVIEW

HISTORY

Koidzumi (1921), Imms (1919), and Cleveland (1923) have given historical reviews of the work done on the Protozoa of termites complete up to the date of their publications, the first two accounts recording the work of various authors, the third the classification given these organisms; it is therefore unnecessary to repeat them. Since Koidzumi's paper was published, Cutler (1921) issued a paper on a flagellate which he named *Pseudotrichonympha pristina* from *Archotermopsis wroughtoni,* though it seems to differ from the other members of the genus enough to make its location there doubtful. Koidzumi noted only one paper of Dogiel's, that of 1916 on Tetramitidae. Dogiel also described *Joenia intermedia* from *Hodotermes mossambicus* in 1916, and in 1917 he recorded *Cyclonympha strobila* from *Coptotermes* species from Japan. This, however, is the same as Koidzumi's *Teratonympha,* so it is likely that Dogiel confused *Reticulitermes* with *Coptotermes*, as may be done where the workers only are obtained. It is made certain that he did this when he states in a later paper that this *Coptotermes* sp. contained *Dinenympha gracilis, Pyrsonympha vertens,* and a *Holomastigotes* species, which are characteristic flagellates of *Reticulitermes.* Both Koidzumi's Japanese paper and Dogiel's Russian paper were published in 1917. While Dogiel's figures are named in Latin, Koidzumi's are not. Therefore the 1921 paper is used as the original of Koidzumi's descriptions, and *Teratonympha* may be a synonym of *Cyclonympha.* Another paper in Dogiel's series (1922) describes some Trichonymphidae from *Hodotermes mossambicus* and *Schedorhinotermes putorius. Pseudotrichonympha introflexibilis* seems to be the same as Grassi's *P. magnipapillosa,* described also from *Schedorhinotermes putorius* in 1917.

Dunkerley (1923) described briefly *Pseudotrichonympha sphaerophora* from *Rhinotermes nasutus* Perthy of British Guiana. Recently Duboscq and Grassé published some brief papers (1923, 1925) on "parasitic Protozoa of the termites of France," describing *Trichomonas trypanoïdes* from *Reticulitermes lucifugus, Janickiella grassii* and *Trichomonas dogieli* from *Kalotermes flavicollis,* and placing

Monocercomonas termitis in the genus *Hexamastix*. These authors found life-cycles of considerable complexity in these organisms, describing young and adult stages as authors have erroneously done in other species.

In several papers published in 1919, 1920, and 1921 Froilano de Mello described some Protozoa from *Leucotermes indicola, Hodotermes viarum,* and two species of *Coptotermes* found in India; identified some of the flagellates figures by Bugnion from *Coptotermes travians, Coptotermes flavus* and *Termitogeton umbilicatus,* as *Trichonympha agilis;* and even stated that Imm's *Trichonympha pristina* was the same as his *T. agilis* from *L. indicola.* He also named several species of *Leidya,* which is a synonym of *Spirotrichonympha.* In *Leucotermes* he discovered *Opalina* and *Balantidium,* and named a new ciliate genus, *Franciella termitis.* These descriptions and determinations need confirmation.

Kofoid and Swezy (1919) published an important series of papers on the flagellates of *Termopsis angusticollis.* The occurrence of certain Trichonymphidae in some termites of Ceylon was mentioned by Bugnion in some of his papers (1911, 1914, 1914*a*). He figured some of these but did not give them any specific names.

The classification adopted by Grassi and Janicki, used in the paper of Cleveland (1923) is the best. Dogiel (1917*c*) gave a classificatory table of those flagellates of termites known to him, founding a new family, the Amoebitidae, to contain his *Myxomonas* and dividing the Trichonymphidae into *Leiotricha* and *Dexiotricha.* Probably he was not aware of the fact that the large group Trichonymphidae had already been split into Trichonymphidae, *s. str.,* Spirotrichonymphidae, and Holomastigotidae; and that the Lophomonadidae were divided into Lophomonadidae, *s. str.,* and Joeniidae. In the following list the classification of Janicki and Grassi is used. The family Streblomastigidae was placed by the authors, Kofoid and Swezy, in the Polymastigina.

SYSTEMATIC LIST OF PROTOZOA LIVING IN TERMITES

CLASS MASTIGOPHORA Diesing

Order POLYMASTIGINA Blochmann

Family TETRAMITIDAE Bütschli

Monocercomonas Grassi, 1881
  M. termitis Grassi, 1892
    Reticulitermes lucifugus Rossi, Italy
    Kalotermes flavicollis Fabricius, Italy
      Grassi and Sandias, 1893
      Grassi and Sandias (Blandford), 1897
      Comes, 1914
      Duboscq and Grassé, 1923 (placed in *Hexamastix*)
Tetratrichomonas Dogiel, 1916 (Paris, 1910)
  T. macrostoma Dogiel, 1916
    Macrohodotermes mossambicus Hagen, British East Africa
  T. termitidis Dogiel, 1916
    Rhinotermes sp., Uganda
Gigantomonas Dogiel, 1916
  G. herculea Dogiel, 1916
    Macrohodotermes mossambicus Hagen, British East Africa
Myxomonas Dogiel, 1916
  M. polymorpha Dogiel, 1916
    Macrohodotermes mossambicus Hagen, British East Africa
Trichomonas Donné, 1837
  T. dogieli Duboscq and Grassé, 1923
    Kalotermes flavicollis Fabricius, France
  T. trypanoïdes Duboscq and Grassé, 1923
    Reticulitermes lucifugus Rossi, France
  T. termopsidis Cleveland, 1924
    Andrews, 1925
    Termopsis angusticollis Hagen, Oregon
      Termopsis nevadensis Hagen, Oregon
Ditrichomonas Cutler, 1919
  D. termitis (Imms) Cutler, 1919
    Archotermopsis wroughtoni Desneux, India
Trichomitus Swezy, 1915
  T. termitidis Kofoid and Swezy, 1919 (probably included in
    Trichomonas termopsidis Cleveland, 1925)
Macrotrichomonas Grassi, 1917
  M. pulchra Grassi, 1917
    Glyptotermes parvulus Sjöstedt, Gold Coast

Devescovina Foa, 1915
  D. glabra Grassi, 1917
    Cryptotermes havilandi Sjöstedt, Nigeria
  D. nova Grassi, 1917
    Neotermes erythraeus Silvestri, Eritraea
  D. lemniscata Kirby, 1926
    Cryptotermes hermsi Kirby, Fanning Island
  D. stereociliata Grassi, 1917
    Glyptotermes parvulus Sjöstedt, Gold Coast
  D. striata Foa, 1905
    Cryptotermes grassii Silvestri [MSS ?], Chile,
      Janicki, 1915
  D. striata var. *hawaiensis* Janicki, 1911
    Neotermes connexus Snyder, Hawaii
Janickiella Duboscq and Grassé, 1923
  J. grassii Duboscq and Grassé, 1923
    Kalotermes flavicollis Fabricius, France
Foaina Janicki, 1915
  F. gracilis Janicki, 1915
    Neotermes connexus Snyder, Hawaii
Caduceia Franca, 1918
  C. theobramae Franca, 1918
    Neotermes gestri Silvestri, Isle of St. Thomas
Paradevescovina Kirby, 1926
  P. nana Kirby, 1926
    Cryptotermes hermsi Kirby, Fanning Island

CALONYMPHIDAE Grassi

Calonympha Foa, 1905
  C. grassii Foa, 1905
    Cryptotermes grassii Silvestri [MSS ?], Chile
Stephanonympha Janicki, 1911
  S. silvestrii Janicki, 1911
    Neotermes connexus Snyder, Hawaii
      Janicki, 1912
      Janicki, 1915
  S. silvestrii var. *cryptotermitis havilandi* Grassi, 1917
    Cryptotermes havilandi Sjöstedt, Nigeria
  S. silvestrii var. *neotermitis erythraei* Grassi, 1917
    Neotermes erythraeus Silvestri, Eritraea
  S. nelumbium Kirby, 1926
    Cryptotermes hermsi Kirby, Fanning Island
Diplonympha Grassi, 1917
  D. foae Grassi, 1917
    Glyptotermes parvulus Sjöstedt, Gold Coast
Microrhopalodina Grassi and Foa, 1911
  M. enflata Grassi and Foa, 1911
    Kalotermes flavicollis Fabricius, Italy

Oxymonas Janicki, 1915
  - *O. granulosa* Janicki, 1915
    - *Neotermes connexus* Snyder, Hawaii
  - *O. parvula* Kirby, 1926
    - *Cryptotermes hermsi* Kirby, Fanning Island

These two genera, *Microrhopalodina* and *Oxymonas*, are closely related. Flagellates resembling them are widely distributed in the *Kalotermitidae* and should probably be allocated to a new family instead of placed in the *Calonymphidae* to which Grassi and Foa (1911) assigned them.

DINENYMPHIDAE Grassi

Dinenympha Leidy, 1877
  - *D. exilis* Koidzumi, 1921
    - *Reticulitermes speratus* Kolbe, Japan
    - *Reticulitermes flaviceps* Oshima, Formosa
  - *D. fimbriata* Kirby, 1924
    - *Reticulitermes hesperus* Banks, California
  - *D. gracilis* Leidy, 1877
    - *Reticulitermes flavipes* Kollar, United States
    - *Reticulitermes lucifugus* Rossi, Italy
      - Leidy, 1881
      - Grassi, 1885
      - Grassi, 1892
      - Grassi and Sandias, 1893
      - Porter, 1897
      - Comes, 1912
      - De Zulueta, 1915
  - *D. leidyi* Koidzumi, 1921
    - *Reticulitermes speratus* Kolbe, Japan
    - *Reticulitermes flaviceps* Oshima, Formosa
  - *D. nobilis* Koidzumi, 1921
    - *Reticulitermes speratus* Kolbe, Japan
  - *D. parva* Koidzumi, 1921
    - *Reticulitermes flaviceps* Oshima, Formosa
    - *Reticulitermes speratus* Kolbe, Japan
  - *D. porteri* Koidzumi, 1921
    - *Reticulitermes speratus* Kolbe, Japan
    - *Reticulitermes flaviceps* Oshima, Formosa
  - *D. rugosa* Koidzumi, 1921
    - *Reticulitermes speratus* Kolbe, Japan

Pyrsonympha Leidy, 1877
  - *P. vertens* Leidy, 1877
    - *Reticulitermes flavipes* Kollar, United States
      - Leidy, 1881
      - Porter, 1897
  - *P. grandis* Koidzumi, 1921
    - *Reticulitermes speratus* Kolbe, Japan
    - *Reticulitermes flaviceps* Oshima, Formosa

P. modesta Koidzumi, 1921
    Reticulitermes speratus Kolbe, Japan
    Reticulitermes flaviceps Oshima, Formosa
(*Pyrsonympha grassii* sp. nov., probably erroneously recorded by De Mello, from *Leucotermes indicola* Wasmann (1921c)
P. affinis Fedorowa, 1923
    "*Coptotermes* from Japan" [=*Reticulitermes*?]
Lophophora Comes, 1910 (probably=*Pyrsonympha*)
    L. vacuolata Comes, 1910
        Reticulitermes lucifugus Rossi, Italy

STREBLOMASTIGIDAE Kofoid and Swezy

Streblomastix Kofoid and Swezy, 1919
    Streblomastix strix Kofoid and Swezy, 1919
        Termopsis angusticollis Hagen, California

Order HYPERMASTIGINA Grassi

LOPHOMONADIDAE Janicki

Eulophomonas Grassi and Foa, 1911
    E. calotermitis Grassi and Foa, 1911
        Kalotermes flavicollis Fabricius, Italy

JOENIIDAE Janicki

Joenia Grassi, 1885
    J. annectens Grassi, 1885
        Kalotermes flavicollis Fabricius, Italy
            Grassi, 1888
            Grassi, 1892
            Grassi and Sandias, 1893
            Grassi and Foa, 1904
            Franca, 1918
    J. intermedia Dogiel, 1917*a*
        Macrohodotermes mossambicus Hagen, British East Agrica
Mesojoenia Grassi and Foa, 1911
    M. decipiens Grassi and Foa, 1911
        Kalotermes flavicollis Fabricius, Italy
Microjoenia Grassi, 1892
    M. hexamitoides Grassi, 1892
        Reticulitermes lucifugus Rossi, Italy
    M. axostylis Cutler, 1920
        Archotermopsis wroughtoni Desneux, India
Joenina Grassi, 1917
    J. pulchella Grassi, 1917
        Porotermes adamsoni Froggatt, Australia

Joenopsis Cutler, 1920
- *J. polytricha* Cutler, 1920
  - *Archotermopsis wroughtoni* Desneux, India
- *J. cephalotricha* Cutler, 1920
  - *Archotermopsis wroughtoni* Desneux, India

Parajoenia Janicki, 1911
- *P. grassii* Janicki, 1911
  - *Neotermes connexus* Snyder, Hawaii
    - Janicki, 1912
    - Janicki, 1915

STAUROJOENINIDAE Grassi

Staurojoenina Grassi, 1917
- *S. mirabilis* Grassi, 1917
  - *Epicalotermes aethiopicus* Silvestri, Eritraea
- *S. assimilis* Kirby, 1926
  - *Kalotermes minor* Hagen, California, Arizona
- *Staurojoenina* sp.
  - *Kalotermes hubbardi* Banks, Arizona

TERATONYMPHIDAE Koidzumi

Cyclonympha Dogiel, 1917 (probably the same as *Teratonympha*)
- *C. strobila* Dogiel, 1917
  - *Coptotermes* sp., Japan (probably *Reticulitermes speratus*)

Teratonympha Koidzumi, 1921 (probably a synonym of *Cyclonympha*)
- *T. mirabilis* Koidzumi, 1921 (probably the same as *Cyclonympha strobila* Dogiel)
  - *Reticulitermes speratus* Kolbe, Japan
- *T. mirabilis* var. *formosana* Koidzumi, 1921
  - *Reticulitermes flaviceps* Oshima, Formosa

SPIROTRICHONYMPHIDAE Grassi

Spirotrichonympha Grassi and Foa, 1911
- *S. flagellata* Grassi and Foa, 1911
  - *Reticulitermes lucifugus* Rossi, Italy
- *S. flagellata* var. *coptotermitis lactei* Grassi, 1917
  - *Coptotermes lacteus* Froggatt, Australia
- *S. flagellata* var. *schedorhinotermitis intermedii* Grassi, 1917
  - *Schedorhinotermes intermedius* Brauer, Australia
- *S. africana* Dogiel, 1922
  - *Macrohodotermes mossambicus* Hagen, British East Africa
- *S. elongata* Grassi, 1917
  - *Schedorhinotermes intermedius* Brauer, Australia
- *S. leidyi* Koidzumi, 1921
  - *Coptotermes formosanus* Shiraki, Formosa
- *S. mirabilis* Grassi, 1917
  - *Porotermes adamsoni* Froggatt, Australia

Leidya Franca, 1916 (synonym of *Spirotrichonympha*)
        Franca, 1918
    L. metchnikovi Franca, 1916 (=*S. flagellata* Grassi and Foa, 1911)
        Reticulitermes lucifugus Rossi, Portugal
        Leucotermes indicola Wasmann, India
            De Mello, 1919*a*, *b*; 1920*b*; 1921*a*, *b*, *c*
    L. annandalei De Mello, 1919
        Leucotermes indicola Wasmann, India
    L. campanula De Mello, 1919
        Leucotermes indicola Wasmann, India
            De Mello, 1919*a*, *b*; 1920*a*, *b*; 1921*a*, *c*
Spirotrichonymphella Grassi, 1917
    S. pudibunda Grassi, 1917
        Porotermes adamsoni Froggatt, Australia

HOLOMASTIGOTIDAE Grassi

Holomastigotes Grassi, 1892
    H. elongatum Grassi, 1892
        Reticulitermes lucifugus Rossi, Italy
        Reticulitermes speratus Kolbe, Japan
        Reticulitermes flaviceps Oshima, Formosa
        Macrohodotermes mossambicus Hagen, British East Africa
            Koidzumi, 1921
            Dogiel, 1922
    H. crassum Dogiel, 1922
        Macrohodotermes mossambicus Hagen, British East Africa
Holomastigotoides Grassi and Foa, 1911
    H. hertwigi (Hartmann) Grassi and Foa, 1911
        Syn: *Trichonympha hertwigi* female. Hartmann, 1910
        Coptotermes hartmanni Holmgren [MSS ?], Brazil
        Leucotermes indicola Wasmann, India
            Guimaraes and D'Andrade, 1924
    H. hartmanni Koidzumi, 1921
        Coptotermes formosanus Shiraki, Formosa
    H. hemigymnum Grassi, 1917
        Coptotermes lacteus Froggatt, Australia
    H. mirabile Grassi, 1917
        Coptotermes sjöstedti Holmgren, French Guinea
Microspironympha Koidzumi, 1921
    M. porteri Koidzumi, 1921
        Reticulitermes flaviceps Oshima, Formosa

TRICHONYMPHIDAE (Kent) Grassi

Trichonympha Leidy, 1877
Trichonympha agilis Leidy, 1877
Reticulitermes flavipes Kollar, United States
Reticulitermes lucifugus Rossi, Italy, Portugal
Leucotermes indicola Wasmann, India
Leidy, 1877
Leidy, 1881
Grassi, 1885, 1892
Grassi and Sandias, 1893
Porter, 1897
Franca, 1916
Grassi, 1917
De Mello, 1919*a*, *b*; 1920*a*, *b*; 1921*a*, *b*, *c*

De Mello (1920) records *T. agilis* from *Hodotermes viarum*, India, from two species of *Coptotermes* obtained near Bombay, and identifies the species figured by Bugnion from *Coptotermes travians*, *Termitogeton umbilicatus* and *Coptotermes flavus*, as *T. agilis*. He bases this chiefly on the general resemblance to the figures of *T. agilis*, and in many cases doubtless mistook *Pseudotrichonympha*.

T. agilis var. *formosana* Koidzumi, 1921
Reticulitermes flaviceps Oshima, Formosa
T. agilis var. *japonica* Koidzumi, 1921
Reticulitermes speratus Kolbe, Japan
T. campanula Kofoid and Swezy, 1919
Termopsis angusticollis Hagen, California
T. leidyi Kent, 1885
Unnamed termite from Tasmania
T. minor Grassi and Foa, 1911
Reticulitermes lucifugus Rossi, Italy
T. magna Grassi, 1917
Porotermes adamsoni Froggatt, Australia
Leidyopsis Kofoid and Swezy, 1919
L. sphaerica Kofoid and Swezy, 1919
Termopsis angusticollis Hagen, California
Leidyonella Frenzel, 1891
L. cordubensis Frenzel, 1891
(?) *Eutermes inquilinus*, Argentina
Gymnonympha Dobell, 1910
G. zeylanica Dobell, 1910
Neotermes militaris Desneux, Ceylon
Pseudotrichonympha Grassi and Foa, 1911
P. hertwigi (Hartmann) Grassi and Foa, 1911
Syn: *Trichonympha hertwigi* male, Hartmann, 1910
Coptotermes hartmanni Holmgren [MSS ?], Brazil
P. hertwigi var. *major* Grassi, 1917
Coptotermes lacteus Froggatt, Australia
P. hertwigi var. *minor* Grassi, 1917
Coptotermes sjöstedti Holmgren, French Guinea

P. grassii Koidzumi, 1921
  Coptotermes formosanus Shiraki, Formosa
P. introflexibilis Dogiel, 1922
  Schedorhinotermes putorius Sjöstedt, British East Africa
P. magnipapillosa Grassi, 1917 (possibly the same as *P. introflexibilis* Dogiel)
  Schedorhinotermes putorius Sjöstedt, Corrakry near French Guinea
P. parvipapillosa Grassi, 1917
  Schedorhinotermes intermedius Brauer, Australia
P. pristina (Imms) Cutler, 1921
  Archotermopsis wroughtoni Desneux, India
P. sphaerophora Dunkerley, 1923
  Rhinotermes nasutus Perty, British Guiana

CLASS SPOROZOA Leuckart

Gregarina Dufour, 1828
  G. termitis Leidy, 1881
    Reticulitermes flavipes Kollar, United States

CLASS CILIATA Bütschli

Order HOLOTRICHA Stein

Opalina Purkinje u. Valentin
  O. termitis De Mello, 1921
    Coptotermes sp., Daman, India
    Leucotermes indicola Wasmann, India

Order HETEROTRICHA Stein

Nyctotherus Leidy
  N. termitis Dobell, 1910
    Neotermes militaris Desneux, Ceylon
  N. fletcheri De Mello, 1919
    Coptotermes sp., Daman, India
    Leucotermes indicola Wasmann, India
Balantidium Claparede and Lachmann em. Stein
  B. termitis De Mello, 1921
    Leucotermes indicola Wasmann, India
Franciella De Mello, 1921
  F. termitis De Mello, 1921
    Coptotermes sp., Pragana, India
    Leucotermes indicola Wasmann, India

LIST OF PROTOZOA LIVING IN TERMITES, ARRANGED ACCORDING TO HOST

Family KALOTERMITIDAE Banks

Subfamily TERMOPSINAE Holmgren

Archotermopsis Desneux
    Archotermopsis wroughtoni Desn., India
        Imms, 1919
        Cutler, 1919, 1920, 1921
            Ditrichomonas termitis Imms
            Joenopsis cephalotricha Cutler
            Joenopsis polytricha Cutler
            Microjoenia axostylis Cutler
            Pseudotrichonympha pristina (Imms) Cutler
Termopsis Heer
    Termopsis angusticollis Hagen, California
        Kofoid and Swezy, 1919*a*, *b*, *c*, *d*
        Cleveland, 1923, 1925*a*, *d*
            Trichomitus termitidis Kofoid and Swezy (probably a *Trichomonas*)
            Streblomastix strix Kofoid and Swezy
            Trichonympha campanula Kofoid and Swezy
            Leidyopsis sphaerica Kofoid and Swezy
            Trichomonas termopsidis Cleveland (probably includes *Trichomitus termitidis* Kofoid and Swezy)
    Termopsis nevadensis Hagen
        Cleveland, 1924, 1925*a*, *d*
            Trichomonas termopsidis Cleveland
                Also seems to have all those of *T. angusticollis*

Subfamily HODOTERMITINAE Holmgren

Macrohodotermes Fuller
    Macrohodotermes mossambicus (Hagen) Fuller, South Africa
        Dogiel, 1916, 1917*a*, 1917*c*
            Tetratrichomonas macrostoma Dogiel
            Gigantomonas herculea Dogiel
            Myxomonas polymorpha Dogiel
            Joenia intermedia Dogiel
            Holomastigotes elongatum Grassi
            Holomastigotes crassum Dogiel
            Spirotrichonympha africana Dogiel
    (?) *Hodotermes viarum* (Smeath.) Hagen, Combiatore, India (*not identifiable*).
        De Mello, 1920*b*, pl. 170, figs. 80–86
            (?) *Trichonympha agilis* Leidy
            (?) *Leidya metchnikovi* Franca
            "Numerous other species."
                These determinations, made only from the figures reproduced, probably are not accurate.

Subfamily KALOTERMITINAE Holmgren

- *Porotermes* Hagen
  - *Porotermes adamsoni* Froggatt, Australia
    - Grassi, 1917
      - *Joenina pulchella* Grassi
      - *Pseudotrypanosoma giganteum* Grassi
      - *Spirotrichonympha mirabilis* Grassi
      - *Spirotrichonymphella pudibunda* Grassi
      - *Trichonympha magna* Grassi
- *Kalotermes* (Hagen) s. str. Holmgr.
  - *Kalotermes flavicollis* Fabr., Mediterranean countries.
    - Grassi, 1885, 1888, 1892
    - Grassi and Sandias, 1893
    - Grassi and Foa, 1904
    - Grassi and Foa, 1911
    - Franca, 1918
    - Duboscq and Grassé, 1923, 1924, 1925
      - *Eulophomonas calotermitis* Grassi
      - *Joenia annectens* Grassi
      - *Mesojoenia decipiens* Grassi
      - *Microrhopalodina enflata* Grassi
      - *Monocercomonas termitis* Grassi
      - *Hexamastix termitis* Duboscq and Grassé
      - *Janickiella grassii* Duboscq and Grassé
      - *Trichomonas dogieli* Duboscq and Grassé
- *Neotermes* Holmgr.
  - *Neotermes connexus* Snyder, Hawaii (erroneously called *Neotermes castaneus* Burm.)
    - Janicki, 1911, 1912, 1915
      - *Devescovina striata* var. *hawaiensis* Janicki
      - *Foaina gracilis* Janicki
      - *Oxymonas granulosa* Janicki
      - *Parajoenia grassii* Janicki
      - *Stephanonympha silvestrii* Janicki
  - *Neotermes erythraeus* Silv., Eritraea
    - Grassi, 1917
      - *Devescovina nova* Grassi
      - *Stephanonympha silvestrii* var. *neotermitis erythraei* Grassi
  - *Neotermes gestri* Silv., Isle of St. Thomas
    - Franca, 1918
      - *Caduceia theobromae* Franca
  - *Neotermes militaris* Desn., Ceylon
    - Dobell, 1910
      - *Gymnonympha zeylanica* Dobell
      - *Nyctotherus termitis* Dobell
- *Cryptotermes* Banks
  - *Cryptotermes grassii* Silv. [MSS ?], Chile
    - Foa, 1905
    - Janicki, 1915
      - *Calonympha grassii* Foa
      - *Devescovina striata* Foa

Cryptotermes havilandi Sjöst., Nigeria
Grassi, 1917
Devescovina glabra Grassi
Stephanonympha silvestrii var. *cryptotermitis havilandi* Grassi
Cryptotermes hermsi Kirby, Fanning Island
Kirby, 1926
Devescovina lemniscata Kirby
Stephanonympha nelumbium Kirby
Paradevescovina nana Kirby
Oxymonas parvula Kirby
Epicalotermes Silv.
Epicalotermes aethiopicus Silv., Eritraea
Grassi, 1917
Staurojoenina mirabilis Grassi
Glyptotermes Froggatt
Glyptotermes parvulus Sjöst., Gold Coast
Grassi, 1917
Devescovina stereociliata Grassi
Diplonympha foae Grassi
Macrotrichomonas pulchra Grassi

Family RHINOTERMITIDAE Froggatt

Schedorhinotermes Silv.
Schedorhinotermes putorius Sjöst., French Guinea
Grassi, 1917
Dogiel, 1916, 1922
Pseudotrichonympha magnipapillosa Grassi
Pseudotrichonympha introflexibilis Dogiel
Probably same as Grassi's species
Tetratrichomonas termitidis Dogiel
Schedorhinotermes intermedius Brauer, Australia
Grassi, 1917
Pseudotrichonympha parvipapillosa Grassi
Spirotrichonympha flagellata var. *schedorhinotermitis intermedii* Grassi
Spirotrichonympha elongata Grassi
Rhinotermes s. str. Hagen
Rhinotermes nasutus Perty, British Guiana
Dunkerley, 1923
Pseudotrichonympha sphaerophora Dunkerley
Rhinotermes sp., Uganda
Dogiel, 1916
Tetratrichomonas termitidis Dogiel
Coptotermes Wasmann
Coptotermes formosanus Shiraki, Formosa
Koidzumi, 1921
Holomastigotoides hartmanni Koidzumi
Pseudotrichonympha grassii Koidzumi
Spirotrichonympha leidyi Koidzumi

  - *Coptotermes hartmanni* Holmgr. [MSS ?], Brazil
    - Hartmann, 1910
      - *Holomastigotoides hertwigi* (Hartmann) Grassi
      - *Pseudotrichonympha hertwigi* (Hartmann) Grassi
  - *Coptotermes lacteus* Froggatt, Australia
    - Grassi, 1917
      - *Holomastigotoides hemigymnum* Grassi
      - *Pseudotrichonympha hertwigi* var. *major* Grassi
      - *Spirotrichonympha flagellata* Grassi
  - *Coptotermes sjöstedti* Holmgr., French Guinea
    - Grassi, 1917
      - *Holomastigotoides mirabilis* Grassi
      - *Pseudotrichonympha hertwigi* var. *minor* Grassi
  - *Coptotermes* sp., Daman, India
    - De Mello, 1919, 1920
      - *Trichonympha agilis* Leidy
      - *Leidya metchnikovi* Franca
      - *Leidya annandalei* De Mello
      - *Opalina termitis* De Mello
      - *Nyctotherus fletcheri* De Mello
  - *Coptotermes* sp., Pragana, India
    - De Mello, 1919, 1920
      - *Trichonympha agilis* Leidy
      - *Leidya metchnikovi* Franca
      - *Leidya annandalei* De Mello
      - *Leidya kempi* De Mello
      - *Franciella termitis* De Mello

- *Reticulitermes* Holmgr.
  - *Reticulitermes flavipes* Kollar, United States
    - Leidy, 1877, 1881
    - Porter, 1897
      - *Dinenympha gracilis* Leidy
      - *Pyrsonympha vertens* Leidy
      - *Trichonympha agilis* Leidy
      - *Gregarina termitis* Leidy
  - *Reticulitermes hesperus* Banks, United States
    - Kirby, 1924
      - *Dinenympha fimbriata* Kirby
      - *Dinenympha* sp.
      - *Pyrsonympha* sp.
      - *Holomastigotes* sp.
      - *Spirotrichonympha* sp.
      - *Microspironympha* sp.
      - *Trichonympha* sp.
  - *Reticulitermes speratus* Kolbe, Japan
    - Koidzumi, 1921
    - (Dogiel, 1917*b*, *Cyclonympha strobila*)
      - *Dinenympha exilis* Koidzumi
      - *D. leidyi* Koidzumi
      - *D. nobilis* Koidzumi
      - *D. parva* Koidzumi
      - *D. porteri* Koidzumi
      - *D. rugosa* Koidzumi
      - *Pyrsonympha grandis* Koidzumi

- *P. modesta* Koidzumi
- *Holomastigotes elongatum* Grassi
- *Microspironympha porteri* Koidzumi
- *Teratonympha mirabilis* Koidzumi
- *Trichonympha agilis* var. *japonica* Koidzumi

Dogiel (1917*b*) describes *Cyclonympha strobila* from a *Coptotermes* sp. from Japan. He also records *Dinenympha gracilis, Pyrsonympha vertens, Holomastigotes* sp. and *Trichomonas* sp. from this termite. Doubtless he had a *Reticulitermes* sp., probably *R. speratus*.

Reticulitermes flaviceps Oshima, Formosa
(Probably a synonym of *R. speratus* Kolbe)
Koidzumi, 1921

- *Dinenympha exilis* Koidzumi
- *D. leidyi* Koidzumi
- *D. parva* Koidzumi
- *D. porteri* Koidzumi
- *Holomastigotes elongatum* Grassi
- *Pyrsonympha grandis* Koidzumi
- *P. modesta* Koidzumi
- *Microspironympha porteri* Koidzumi
- *Teratonympha mirabilis* var. *formosana* Koidzumi
- *Trichonympha agilis* var. *formosana* Koidzumi

Reticulitermes lucifugus Rossi, Mediterranean countries
Grassi, 1892
Grassi and Sandias, 1893
Comes, 1910, 1912, 1914
Franca, 1918
De Zulueta, 1915 (*Dinenympha*)
Duboscq and Grassé, 1925 (*Holomastigotes*)

- *Dinenympha gracilis* Leidy
- *Holomastigotes elongatum* Grassi
- *Microjoenia hexamitoides* Grassi
- *Monocercomonas termitis* Grassi
- *Spirotrichonympha flagellata* Grassi
- *Trichonympha agilis* Leidy
- *Lophophora vacuolata* Comes (*Pyrsonympha*)
- *Leidya metchnikovi* Franca (*Spirotrichonympha*)
- *Trichomonas trypanoides* Duboscq and Grassé

Leucotermes Silv.

Leucotermes indicola Wasm., India
De Mello, 1919*a*. *b*; 1920*b*; 1921*a*, *c*
Guimaraes and D'Andrade, 1924

- *Trichonympha agilis* Leidy
- *Leidya metchnikovi* Franca
- *L. annandalei* De Mello
- *L. kempi* De Mello
- *L. campanula* De Mello
- *Pyrsonympha grassii* De Mello
- *Opalina termitis* De Mello
- *Balantidium termitis* De Mello
- *Nyctotherus fletcheri* De Mello
- *Franciella termitis* De Mello
- *Holomastigotoides hertwigi* (Hartmann) Grassi and Foa

Some of these determinations are very doubtful.

GENERAL HISTORY OF THE STUDY OF RELATED FLAGELLATES

Most closely related to the Staurojoenidae are the Lophomonadidae and Joeniidae. The Teratonymphidae are close in some features of structure, and some general resemblances may be traced among the Spirotrichonymphidae, Holomastigotidae, and Trichonymphidae. The earlier and many of the later studies were undertaken, not with a view to the accurate determination of structure, but rather for the purpose of recording the existence of previously unknown organisms. *Trichonympha* has been studied most carefully and accurately by Kofoid and Swezy (1919), who worked on *T. campanula* from *Termopsis angusticollis*. *Lophomonas,* the only closely related flagellate not found in termites, was worked up thoroughly by Janicki (1910), who gave a full account of the phenomena of structure and division. The most detailed account of *Joenia annectens* was given by Grassi and Foa (1904), but it omits some of the finer points. Dogiel (1917*a*) described a second species, *J. intermedia.* Five other genera of Joeniidae have been founded. *Teratonympha* was described by Koidzumi (1917) in Japanese, by Dogiel as *Cyclonympha* in the same year in Russian, later by Koidzumi (1921) in English (including an account of division). In this paper Koidzumi also wrote in some detail on *Pseudotrichonympha.* These are the most complete accounts of Hypermastigina, though many others, recorded in the list of species, have been written.

COMPARATIVE MORPHOLOGY OF THE HYPERMASTIGINA

1. The hypermastigote flagellates are of relatively large size, from 40 to 450 microns, with the length usually above 100 microns.

2. The numerous flagella are commonly located in the anterior region of the body, but may cover the surface. In some of the simplest forms (Lophomonadidae, Joeniidae) these are arranged in a tuft at the anterior end. In *Staurojoenina* they are still all in the anterior portion of the body, but are arranged in four longitudinal bundles. A more general distribution over the anterior portion of the body occurs in *Trichonympha,* and in *Pseudotrichonympha.* The flagella of the Spirotrichonymphidae and the Holomastigotidae also arise from spiral lines covering the whole or most of the body.

Development seems to have progressed from an apical tuft, at first numbering only a few, to a more general distribution over the surface in spiral or longitudinal lines. *Staurojoenina* is intermediate in this development.

3. The flagella arise from basal granules which are imbedded in a plate-like structure of dense, homogeneous protoplasm. This plate, best developed as such in the Lophomonadidae and Joeniidae, becomes more dispersed with the distribution of flagella. It is a very definite refractive structure in *Joenia, Joenina,* and *Joenopsis,* and is split into a series of bands in *Staurojoenina.* The ridges of *Joenopsis* while more superficial, bear some resemblance to the peripheral bands of *Staurojoenina.* In the higher forms there is an axial column in the head which in whole or in part constitutes the centroblepharoplast. From a granule-bearing zone of this the anterior flagella take origin, and in addition there may be body flagella arranged in circular, longitudinal, or spiral series.

These structures, which serve as foundation supports for the vigorous flagella, suggest the plates at the base of the undulating membranes, membranelles, and cirri of ciliates. The undulating membranes of ciliates are composed of rows of united cilia arising from lines of basal granules imbedded in the outer zone of an ectoplasmic thickening, the basal lamella, Membranelles and cirri are supported by similar basal plates from which fibers may run deeper into the protoplasm.

4. As in other flagellates there is a blepharoplast, or centroblepharoplast where there is not a separate centrosome; this is often very different from the typical granule in the lower families. In the Joeniidae there is a granule or rod located anteriorly, as are the granule of *Joenia,* the two curved rods of *Joenina,* and the granular body of *Parajoenia.* In *Joenopsis* no separate blepharoplast is described, but it is possible that it is included in the supporting piece at the base of the head flagella.

In *Staurojoenina* the centroblepharoplast is divided into four parts to correspond to the quadrupling of the neuromotor organelles. These four parts form a column which extends from the tip to the nucleus; the nuclead portions are referred to as centroblepharoplast bands. In the Spirotrichonymphidae, Trichonymphidae, and Teratonymphidae there is a tube in place of this quadripartite column; the tube may be solid or composed of strands. This tube may also be called the centroblepharoplast.

5. An axostyle is frequently present, especially in the lower forms. This is well developed in the Lophomonadidae and Joeniidae, where it may be a straight rod from the blepharoplast to the posterior end of the body or may flare out to enclose the nucleus, as the calyx of *Lophomonas.* It may also be connected by strands to the nucleus (*Joenia*), or fanned out anteriorly into filaments (*Joenina*).

In the higher forms there is not so definite an axostyle. There are, however, filaments which embrace the nucleus and extend backward in the endoplasm. These are connected to the flagellar plates and ultimately to the centroblepharoplast. Filamentous structures in the endoplasm are discernible in the Teratonymphidae, Spirotrichonymphidae, Holomastigotidae, and *Trichonympha,* and these are all probably equivalent to each other and to the body filaments of *Staurojoenina,* all, at least in part, representing the axostyle.

6. Parabasal bodies, which are universally present in the Polymastigina, are found in the lower genera of Hypermastiginia; and structures which represent them are found in the higher species of the order. The existence of this apparatus is almost universal among parasitic flagellates, and though the form and method of its origin is various, it is always associated with the locomotor organelles, and generally attached by a thread to the blepharoplast.

In the Lophomonadidae and Joeniidae the parabasal apparatus, generally well developed, consists of numerous rod-like or leaf-like bodies attached to a filament. Thus in *Lophomonas* the nearly spherical structure of radially arranged rods surrounds the calyx; in *Joenia* and *Mesojoenia* a string of leaf-like bodies in single or double row surrounds the axostyle back of the nucleus and connects to the blepharoplast by the *"regolo";* and in *Joenopsis* there are two longitudinal bands leaving the "supporting piece" or centroblepharoplast, and to these many short, clavate bodies are attached. *Joenina* possesses a parabasal in the form of a ribbed cage enclosing the nucleus, and *Parajoenia,* which may not really belong to the Joeniidae, has two stout curved bodies near the nucleus.

Parabasals are less definite above the Joeniidae, but their presence is most likely. Janicki (1915, p. 652) states that the rounded corpuscles along the spiral lines of *Spirotrichonympha* represent parabasal bodies, and that the corbule of *Trichonympha* is a very much modified parabasal apparatus, having, also, homologies with the axostyle. In the latter statement he agrees with Grassi. The parabasal apparatus of *Staurojoenina* is probably represented in the major body filaments, which are composed of fused strands. It is more difficult to trace parabasals in the genera *Holomastigotoides, Pseudotrichonympha, Microspironympha, Teratonympha,* and others of the groups to which these belong. With the distribution of flagella over the body surface, the parabasal apparatus may be distributed instead of localized into a single mass as in the Polymastigina, where there are few flagella all connected to the blepharoplast, and the Joeniidae, where the flagella are all in a polar tuft. *Microjoenia* and *Spirotrichonympha* show this dispersal into relatively large granules along the lines from which the flagella originate. The granular bands or rods of *Trichonympha* are also associated with the flagellated zone, as are the filaments of *Staurojoenina* which connect to the peripheral bands. The endoplasmic filamentous structures of the Teratonymphidae (Koidzumi, 1921), which are covered with deeply staining granules, may also have parabasal homologies. In *Pseudotrichonympha* the other minute granules, forming a zone near the basal granules of the flagella, may be the parabasal apparatus.

These parabasal structures are preserved in fixation by corrosive sublimate and acetic acid and stained well by iron alum haematoxylin, which technique gives excellent results and has been the one most used in the studies of these flagellates. They are readily demonstrable as refractive structures in living organisms.

7. The nucleus is often connected to or suspended from the centroblepharoplast. The calyx encloses the nucleus in *Lophomonas;* no other direct connections have been made out. Among the Joeniidae nuclear connections have been found in some but not in all species. The *"nastrino"* of *Joenia* suggests a band suspending the nucleus. *Joenina* possesses two filaments which arise from the blepharoplast and approach the nucleus. In *Staurojoenina* there are two bands impinging very definitely on the nuclear membrane and certainly not forming a sac such as has been found in some forms. Cutler finds two such suspensory filaments in *Pseudotrichonympha pristina,* which possibly belongs to another genus and may be closer to the Joeniidae than to the Trichonymphidae. These bands are attached as are those of *Staurojoenina.* According to Koidzumi, a sac hanging from the axial column envelops the nucleus of *Teratonympha.* The appearance of this structure in Koidzumi's figures is much like that of *Staurojoenina,* and the sac, if such it is, is possibly a development of the bands. A similar sac is described by Koidzumi in *Pyrsonympha.* In the Spirotrichonymphidae, where there is an anterior tubular

centroblepharoplast as in *Pseudotrichonympha* and *Trichonympha*, this may extend as an area of dense protoplasm to the nucleus. Also in *T. agilis* var. *japonica*, as described by Koidzumi (1921), there is a columnar mass of protoplasm, consisting of a matrix in which granules are imbedded, extending from the apex of the "bell" to the nucleus. A similar structures exists in *Trichonympha campanula*.

The terms rhizoplast has been used for the strand connecting the blepharoplast to the centrosome, which lies on the nuclear membrane, or attaches the centroblepharoplast to the nucleus. Some of these bands connecting the centroblepharoplast to the nucleus in Hypermastigina are therefore homologous to rhizoplasts, and the term rhizoplast bands is applicable in *Staurojoenina*, perhaps also in *P. pristina*, *Teratonympha*, and *Pyrsonympha*.

8. There are some differentiated intracellular structures which it is difficult to place in the usual categories. Thus in *Joenia* there are the myophanes attached to the apical thickening at the terminus of the axostyle, and the "lamina," an anterior quadrangular area of dense protoplasm extending between the flagellated zone and the top of the axostyle. Dogiel (1917*a*) finds in *Joenia intermedia* two "rulers" forming, he says, a supporting frame for the interior collar. One of them he homologizes with the rules (*regolo*) of *J. annectens;* the other is similar. These may be parabasal bodies—he finds no others—but if so, his organism becomes generically different from *J. annectens*. The complicated hood (*cappuccio*) of *Joenina*, a prolongation of the lateral part of the flagellated zone, suggests the lamina of *Joenia*, but the long threads attaching to the blepharoplast, which only doubtfully represent the bands running to the nucleus, are peculiar to this organism.

When we come to *Staurojoenina* we find several peculiar structures. The ctenofilaments may be a development of the axostyle to add to the skeletal structures of the head. They send long filaments into the axostylar major body filaments. The accessory filaments may be specially developed for support. The peripheral bands are unique structures which evidently develop from the centroblepharoplast bands. Since they give origin to the flagella they evidently represent the flagellated zone of thickened ectoplasm. The filamentous prolongations of these peripheral bands seem to correspond functionally, as well as in general form, with the ctenofilaments; certain of the inner ones, at least the long ones which enter into the major body filaments, possibly, as stated before, represent the parabasal bodies.

The complicated system of fibers and myonemes discovered by Kofoid and Swezy in *Trichonympha campanula* and *Leidyopsis sphaerica* has as yet been discovered only in their organisms. However, no other observations on the flagellates of termites have been as careful as those of Kofoid and Swezy. The systems are not present in *Staurojoenina* and Koidzumi says he could not find them in *T. agilis* var. *japonica.*

9. The surface of the body is viscid, so that microörganisms and food particles adhere to it. It does not seem to be viscid at all times, but is very much so at other times. Particles of wood which are being ingested adhere to the surface, others are thrown off. Swezy (1923), observing *Leidyopsis* sp., suggests that the particles of wood may be held firmly by secretions of the ectoplasm. In the activities shown in figure E the end of the arm of a flagellate certainly held very firmly to the surface of a piece of wood.

Microörganisms commonly adhere by one end to the body surface where, especially in fixed material, they simulate flagella. The facts that those adhering to an organism are sometimes all of one kind, which may be peculiar to that organism, and that they occur in approximately equal numbers on different individuals, have led to some erroneous interpretations. They are common on the bodies of Tetramitidae, Dinenymphidae, Calonymphidae, less common on certain of the Hypermastigina. These microörganisms have often been called flagella in many genera; namely in *Dinenympha, Devescovina, Caduceia, Stephanonympha, Oxymonas, Joenia, Joenopsis,* etc. The immobile short "cilia" which Grassi described on *Joenia annectens* (Grassi and Foa, 1904); the "flagella" scattered over the body of *Joenopsis polytricha* (Cutler, 1920, p. 386); which "may be seen projecting in all directions from the body edge and are in continuous movement, which is, however, so erratic and feeble that it may be described as quivering"; the bristle-like "flagella" of *Parajoenia grassi* (Janicki, 1915, p. 591), and possibly also some of the anterior flagella of this organism; the relatively large, stout "flagella" distributed over the body of *Spirotrichonymphella pudibunda* (Grassi, 1917, p. 44); the bacteria shown by Dogiel on certain forms (?) of *Spirotrichonympha africana* (1917*c*); all these are very probably microörganisms adhering by one end. On many other described species such organisms were not noted: *Joenia intermedia, Joenopsis cephalotricha, Joenina pulchella, Teratonympha mirabilis,* most species of *Spirotrichonympha, Holomastigotoides,* all species of *Pseudotrichonympha, Trichonympha,* and others.

NUCLEAR STRUCTURE OF THE FLAGELLATES OF TERMITES

It is possible that the flagellates of termites were evolved from a simple polymastigote ancestor which originated from soil, and that the marvelous speciation has taken place entirely in the intestines of the insects (cf. Cleveland, 1923, p. 216). It is most interesting, therefore, to survey the forms which have been developed and to compare the nuclear structure of the different species. The nucleus, being least subject to adaptive modifications, will therefore, it is expected, be found to be constant in character. The term endosome (cf. Kirby, 1924) is used for the chromatic body in the nucleus (referred to variously as *Binnenkörper,* karyosome, nucleolus), since its use does not indicate the precise nature of the body. Sometimes it contributes to the formation of the chromosomes, sometimes it disintegrates like the nucleolus of Metazoa. In the former case the term karyosome will be used, in the latter the term nucleolus.

In the free-living polymastigote *Collodictyon* the vesicular nucleus contains a large endosome and peripheral chromatin material distributed in fine granules in the outer alveolar zone. According to the description of Rhodes (1919), the karyosome contributes to the formation of the chromosomes, but Bělař (1921) finds that it disintegrates. Nuclei in which there is a central karyosome, often large, and little outer chromatin are commonly found in parasitic trichomonads. In these most of the chromatin has been collected into a central mass from which the chromosomes organize (cf. *Trichomonas augusta,* Kofoid and Swezy, 1915; *Chilomastix mesnili,* Kofoid and Swezy, 1920; *Monocercomonas,* Jollos, 1911; *Pentatrichomonas,* Kofoid and Swezy, 1923; *Hexamitus,* Swezy, 1915; *Trichomitus parvus,* Swezy, 1915; and many others).

In accord with these trichomonads living in other animals, those living in termites have karyosomes which contain most of the chromatin material. That of *Myxomonas* often consists of large round grains of chromatin (Dogiel, 1916); that of *Trichomitus termitidis* may be resolved into fairly uniform, rounded granules. Probably sufficient careful observation would resolve many of the large trichomonad karyosomes into granules. *Gigantomonas herculea* (Dogiel, 1916) differs from the usual type in having a karyosome not of massed chromatin, but distributed in fine granules.

The usual type of trichomonad nucleus occurs in the Calonymphidae. The tetramitid *Devescovina* has a central mass of granular chromatin (Janicki, 1915; Kirby, 1926). In one form of *Stephanonympha* and in *Calonympha* there is a small vesicle-enclosed "*Binnenkörper*" in addition to this karyosome, and this disappears in division.

In the Dinenymphidae there are nucleoli which disappear during nuclear changes and are associated with chromatin material distributed on an alveolar framework. These plastin nucleoli are not granular like trichomonad karyosomes, but show an alveolar structure.

In the Hypermastigina we meet with nuclei of the same general type. *Joenia* and *Joenina* have most of the chromatin material concentrated into large karyosomes with small chromatin granules in the outer zone. The nucleus of *Joenopsis* closely resembles that of *Staurojoenina;* the large granular karyosome occupies a large volume within the nucleus, the outer zone is clear. That of *Teratonympha* is similar, save that there is a small nucleolus present. A large spherical mass composed of a few large or many small chromatin masses occupies a large part of the nucleus of *Trichonympha,* and is surrounded by a clear area. Grassi and Koidzumi do not refer to nucleoli in their species of *Trichonympha,* but Kofoid and Swezy find a "heterochromosome," which divides and lags in mitosis. In *Pseudotrichonympha* the resting nuclei are similar to those of *Trichonympha,* irregularly shaped, variously sized chromatin masses in a matrix of plastin, surrounded by a clear space. Cutler (1921) refers to a nucleolar structure in *P. pristina.* The type is essentially the same in the Holomastigotidae and Spirotrichonymphidae.

The nucleus of *Oxymonas* much resembles that of the Euglenoidina, having an endosome which in mitosis constricts and is distributed to the daughter nuclei.

The nuclear structure of these flagellates is thus fundamentally the same, the chromatin being more or less concentrated into a central granular mass, the outer zone clear or containing some granules, and a separate plastin nucleolus sometimes present but more frequently absent. *Staurojoenina* is typical.

NUCLEAR DIVISION IN THE FLAGELLATES OF TERMITES

In his review of the process of nuclear division in flagellates Janicki (1912) distinguishes two main types: one for those which possess an axostyle or its homologue—most of the Polymastigina and Hypermastigina; another for those which lack such a structure—the Protomonadina and free-living flagellates. The members of the former group divide with what he calls (1912, p. 30) the Grassi type of nuclear division, which is characteristic of *Trichomastix, Trichomonas, Lophomonas, Joenia, Parajoenia, Devescovina, Calonympha,* and *Stephanonympha.* These lack centrioles within the nucleus but have, in division, an extranuclear central spindle, the paradesmose of Kofoid and Swezy, often with centrosomes at the poles; the nuclear membrane remains intact, and within it a linin spindle appears. The latter group possesses an intranuclear spindle developed from centrioles which may be located within the karyosome of the vesicular nucleus. It seems likely, however, that nuclear division in flagellates is fundamentally of one type, characterized by the extranuclear centrosomes and the paradesmose, and that the presence of centrioles within the nuclear membrane (cf. Bělař, 1921, *Chilomastix, Collodictyon;* Bělař, 1915, *Astasia, Rhynchomonas;* Bělař, 1916, *Chilomonas, Trypanoplasma*) is doubtful. Bělař (1916) shows a paradesmose in *Monocercomonas,* Kofoid and Swezy (1920) in *Chilomastix;* and evidence has been found for the existence of a paradesmose in Euglenoidina and Dinoflagellata (Hall, 1923, *Menoidium, Euglena agilis*; Hall, 1924, *Oxyrrhis*).

Janicki supposes that the axostyle is a remnant of the paradesmose, which always has a fibrillar structure though, by reason of small diameter and deep staining, this is often not seen. Prowazek (1904) and Dobell (1909) also regarded the axostyle of trichomonads as derived from the chromatic fiber connecting the blepharoplasts. However, there has been no indubitable proof that this is so, and Kofoid and Swezy, who regard the axostyle as an intracytoplasmic flagellum, state definitely that the paradesmose fades away without leaving any special organ as its successor. This it certainly does in *Staurojoenina.*

Since Janicki's work several important accounts of nuclear division in these flagellates have appeared, written by De Zulueta (1915), and Kirby (1924), on *Dinenympha;* Dogiel (1916) on *Myxomonas;* Dogiel (1917*a*) on *Joenia;* Cutler (1919) on *Ditrichomonas;* Kofoid and Swezy (1919) on *Streblomastix, Trichomitus, Trichonympha,* and *Leidyopsis;* Cutler (1920) on *Joenopsis;* Cutler (1921) on *Pseudotrichonympha pristina;* Koidzumi (1921) on *Trichonympha, Pseudotrichonympha, Teratonympha, Microspironympha, Holomastigotoides, Pyrsonympha,* and *Dinenympha;* Dogiel (1922), on *Spirotrichonympha* and *Pseudotrichonympha.* Janicki wrote on *Lophomonas* (1910), later (1915) on *Devescovina, Parajoenia, Stephanonympha, Calonympha,* and *Oxymonas;* Grassi and Foa (1904) on *Joenia;* Foa (1904) on *Trichonympha.* These fairly cover the field of the termite flagellates so far known, but are not all as thorough as is desirable.

The observations made on *Staurojoenina* may be summarized here. The nucleus is suspended in the anterior portion of the body by the two rhizoplast bands. It is spherical, the chromatin gathered in a granular mass withdrawn from the membrane. At the onset of mitosis this granular mass disperses, small round granules coming to occupy the entire space within the membrane. These granules aggregate into threads which are probably separate from the first and in the prophase split lengthwise. Meanwhile a granular area appears between the nuclear ends of the rhizoplast bands, which spread apart. In this granular area fibers appear attached at the ends to the bands, and enlarging to a large fibrillar paradesmose which is round in cross-section, in longitudinal section shaped like a thick planoconcave lens, and sunk into an anterior depression of the nuclear membrane.

The split chromosomes shorten and the split disappears for a time. They then become threads again and separate into loops or V's, finally pulling apart at the apex. A feeble spindle, consisting chiefly of fibers attaching the chromosomes to the paradesmose poles, exists within the nuclear membrane, which, as is usual, remains intact and definite through the whole process. The chromosomes, still beaded in appearance, converge to the poles, to which they seem to be attached by the spindle fibers. The neuromotor system divides into two portions which remain parallel but widely separate. An anterior depression initiates fission of the body, which seems to take place rapidly. The remnant of the paradesmose persists for some time after plasmotomy, somewhat spread proximally and drawn out distally. The paradesmose does not elongate greatly as in some (*Joenia*) but remains straight above the nucleus, dividing in the middle. Mitosis in *Staurojoenina* is thus in all essential details of the type described by Kofoid and Swezy (1919*a*) in *Trichonympha.*

Few conclusions can be drawn from the incomplete knowledge we at present possess regarding division in these flagellates, but several generalities can be gathered from the descriptions and some probabilities suggested.

1. The occurrence of a paradesmose is almost universal. Where this has not been described (*Joenopsis, Pseudotrichonympha pristina, Joenia intermedia*) further investigation is desirable. This paradesmose is a stout rod, sometimes short, in the Polymastigina, but in the Hypermastigina it becomes a large fibrillar structure.

2. The paradesmose unites the centrosomes where these are separately present. It unites the centroblepharoplasts where the centrosome is not separated. In some of the tetramitids the centrosome migrates from the centroblepharoplast at the time of division. In the lower Hypermastigina there may be a small centroblepharoplast granule or elongated body, but in the higher forms it is more complex, consisting of strands or a tube. Where it is so complex it may split and spin out a paradesmose between its lower edges, or possibly (*Teratonympha, Pseudotrichonympha grassii*) a centrosome may sometimes separate from it.

The paradesmose sinks into a depression of the nucleus in some species, as in *Joenia, Staurojoenina,* cysts of *Lophomonas,* and less so in *Devescovina.* In others it lies very close against the membrane.

3. The chromatin is usually collected into a central granular mass. The blocks of chromatin break up into small round granules which fill up the intranuclear space. These granules then organize into rounded or thread-like chromosomes, possibly at first in a continuous spireme, but more probably separate from the beginning. They most likely spilit longitudinally in the prophase; though some records of transverse division have been given, this was probably due to misinterpretation, the final separation of the loops or V's so appearing (*Joenia annectens, J. intermedia* (?), *Pseudotrichonympha grassii*).

4. Intranuclear spindle fibers have not been described in many species, but a fairly well developed spindle is present in the Dinenymphidae and *Trichonympha,* and a few fibers in *Staurojoenina.* These spindles consist of a few fibers extending from the chromosomes to the poles of the paradesmose and generally do not appear between the groups of separated chromosomes, that is, they consist mostly of traction or chromosome fibers. Probably such a spindle occurs more commonly than has been reported, for intranuclear fibers are usual in the parasitic flagellates which have been critically studied.

5. The new organelles originate by outgrowth from the neuromotor centers. This is true of flagella in the Tetramitidae (cf. *Trichomitus termitidis*), where they are few, and in the Hypermastigina where there are many (cf. *Lophomonas, Staurojoenina*). The old flagella are usually retained, but in *Lophomonas* the whole bundle is discarded. Not much light is thrown on the origin of new structures in published work on these flagellates. In *Trichonympha* and other similar forms the neuromotor system separates into two parts, thus leaving half of each new animal unprovided, but this gap soon closes over by rounding up and outgrowth. In *Staurojoenina* the four bands separate and each daughter for a time possesses only two, which become opposite. The new ones grow opposite to each other between these. Cutler suggests that the axostyles probably split longitudinally in *Joenopsis*, but this is without proof; probably axostyles in general are discarded (as in *Joenia, Dinenympha*, etc.) and new ones form by outgrowth, or possibly, sometimes, from the paradesmose. Generally either one or both parabasals grow anew, but sometimes splitting is described (*Devescovina, Parajoenia, Ditrichomonas*); these records need confirmation, for the authors' observations were in no case extensive enough to furnish proof.

SYSTEMATIC POSITION

The three specimens of *Staurojoenina*, those from *Kalotermes minor*, those from *Kalotermes hubbardi*, and *S. mirabilis* from *Epicalotermes erythraeus*, resemble each other very closely. Because of the incompleteness of Grassi's account it is impossible to compare them in all particulars. In internal structure they are all very much alike, but they seem to differ in size and number of flagella.

The measurement used as most constant is that from the expanded tip of the centroblepharoplast to the posterior end of the nucleus, though there was a slight variation due to the swinging of the nucleus out of position and the slight difference in nuclear diameter. It seemed more convenient to take the posterior instead of the anterior end of the nucleus, however. Measurements of Grassi's figures give *S. mirabilis* a range from 108 to 117 microns in total length, a nuclear diameter of 13–15 microns, and a measurement from tip of centroblepharoplast to posterior end of nucleus of 44–53 microns. *Staurojoenina* from *Kalotermes minor* is somewhat larger, the total length 110–160 microns, the nuclear diameter 16–17 microns, and the third measurement 57–65, usually 60 microns. This larger size is constant and because of it, together with the fact that the hosts are widely separated, it seems best to assign it a specific name *Staurojoenina assimils,* which is preferable to a variety name.

Staurojoenina sp. from *Kalotermes hubbardi* has measurements comparable to those of *S. mirabilis,* being, therefore, smaller than *S. assimilis.* It also seems to have fewer flagella than this species and for this reason it is best to distinguish it from *S. assimilis* and leave the question open of its identity with *S. mirabilis.* The associated flagellates of *S.* sp., *Caduceia* sp.(?), and a tetramitid, differ from those of *S. assimilis*—namely, *Devescovina, Oxymonas,* and a small tetramitid.

Staurojoenina assimilis sp. nov.

Length of body varies from 105 to 190 microns, averaging 142 microns. Distance from expanded tip of centroblepharoplast to posterior edge of nucleus almost constantly 60 microns, with some variation from 57 to 65 microns. Body shape variable, usually campanulate with anterior region a convex, broad-based cone. Flagella numbering several hundred in a group lie in four bands occupying the sides of the cone. Each band is inserted in a groove and between the grooves are prominent lobes. Flagella originate from four series of about nine ray-like peripheral bands connected anteriorly to the quadripartite centroblepharoplast; these together form a quadrangular prismoidal column with slightly rounded sides, tapering anteriorly. Each part of the centroblepharoplast continues posteriorly in an axial band; two of these attach to the spherical nucleus. The lobes are supported by four series of about twenty ctenofilaments each, each series in the right side of a lobe, and by filamentous prolongations of the peripheral bands in the left side. These filaments continue as endoplasmic fibers back to and beyond the nucleus, and the inner ones are united to form four stout, major, body filaments which continue to the posterior end of the body. The periplast is marked with small, rod-like striations arranged in groups not all parallel to one another, but in general longitudinal. Food consists of particles of wood ingested through the body wall.

Host.—*Kalotermes minor* Hagen from California and Arizona.

SUMMARY

1. *Staurojoenina assimilis* has a complex quadipartite neuromotor system, which is described in the diagnosis, and a series of peculiar cuticular rods sculpturing the body surface.

2. Sometimes one or more of the four bundles of flagella are introverted, and may move in the cytoplasm. These may be thrust out beneath the pellicle as long arms, or may break out through the body wall.

3. The nucleus resembles that of the flagellates of termites in general, having a firm nuclear membrane and a large central mass of chromatin blocks, leaving a clear space occupied by two series of alveoli beneath the nuclear membrane.

4. The animal swims and clears its way by means of the four bands of flagella. In degeneration the body becomes much vacuolated and long processes, some of them simulating pseudopodia, appear from various parts of the body surface.

5. The food usually consists of small particles of wood, sometimes larger particles, which crowd the postnuclear cytosome. These seem to be ingested through the body wall with the aid of some pseudopodial activity. Spirilla and small flagellates may be enclosed in vacuoles in the body, especially in flagellates from termites fed on filter paper.

6. Nuclear division conforms to the Grassi type. There is a large. fibrillar paradesmose between the nuclead ends of the rhizoplast bands; astral rays extend from the ends of this into the cytoplasm; the 24 chromosomes split longitudinally in the prophase and separate into V's in the metaphase; and some chromosomal fibers constitute an intranuclear spindle.

7. The paradesmose parts in the telophase and degenerates. The body splits longitudinally.

8. Each new individual possesses two flagellar bands and associated structures. After a time new flagella grow and new body filaments appear to complete the number four.

9. An eight-celled somatella, probably an animal in multiple fission, was observed once, and binucleate animals with the other organelles of a single individual occur.

10. A complete systematic list of the Protozoa of termites, and a list of the protozoan infections of the termites studied, is given.

11. A comparative study of the structures and nuclear division of the flagellates of termites is made, with *Staurojoenina* as a basis.

12. The bibliography is, as far as possible, complete for the special papers to date dealing with the Protozoa living in termites.

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EXPLANATION OF PLATES

All figures of *Staurojoenina assimilis* sp. nov. from *Kalotermes minor* Hagen.

PLATE I

Fig. 1. Entire animal showing the flagella, nucleus, body filaments, and cuticular striations. × 410.

Fig. 2. Similar, showing some neuromotor structures and appearance of cytoplasm. × 410.

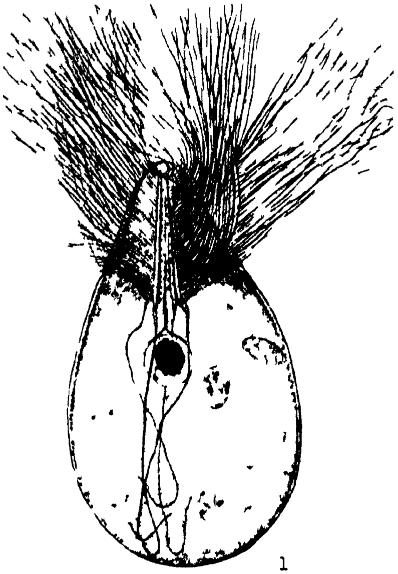
Fig. 3. An optical section through the anterior portion of the body. × 410.

Fig. 4. An enlargement of the anterior portion of the body, showing neuromotor structures. For terminology refer to text figure B. × 800.

Fig. 5. An optical section through the anterior protoplasmic lobes, showing the "square," the origin of the flagellar bundles, the "cross" formed by the bands of the centroblepharoplast, and the deeply staining granules within the square. × 800.

Fig. 6. The rod-like cuticular striations. × 1500.

Fig. 7. A diagram to show how the nucleus is suspended by the crossing rhizoplast bands.



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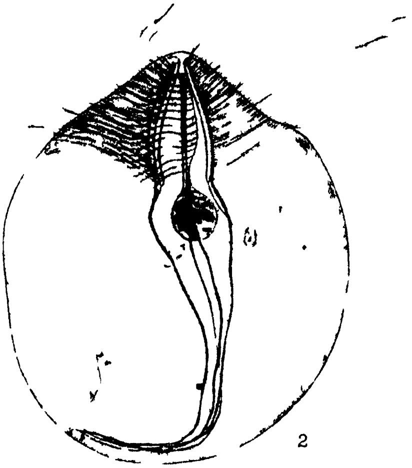

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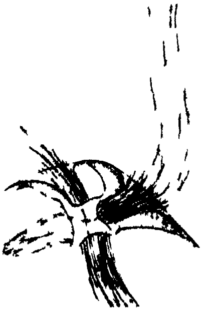

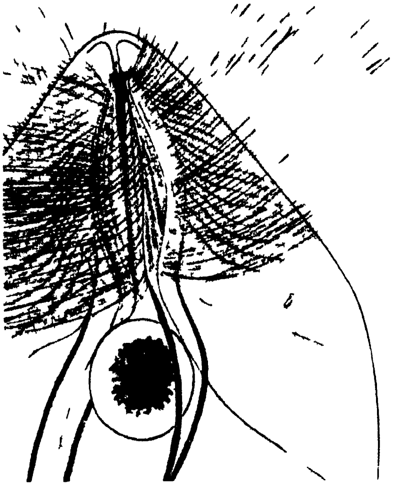

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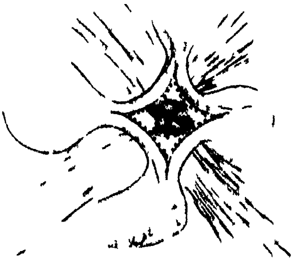

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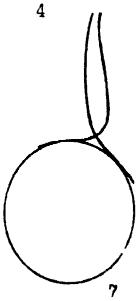

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PLATE 2

Fig. 8. A maceration. On the right the flagella are torn loose from the peripheral bands, which are turned back. The nucleus is suspended by the rhizoplast bands. The coarsely granular protoplasm within the square is indicated. × 800.

Fig. 9. A diagram, drawn from life, to show the structure and attachment of one of the series of peripheral bands. This lies beneath the nucleus; were it turned so as to be above, the largest band would be on the right. There are nine, successively narrower from right to left, each prolonged into a filament. × 800.

Fig. 10. A semidiagrammatic camera lucida drawing of the anterior portion of the body, to show how the flagella are inserted in the outer face of the plates and how the basal granule series is connected to the centroblepharoplast. × 1500.

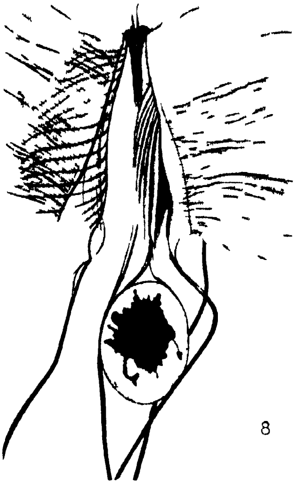
Fig. 11. A maceration of the peripheral bands, showing the connection of flagella and the connection to the centroblepharoplast. × 1500.

Fig. 12. Semidiagrammatic camera lucida sketch of the peripheral bands and ctenofilaments. × 1500.

Fig. 13. Origin of flagella. × 1500.

Fig. 14. A maceration, showing ctenofilaments, peripheral bands, origin of flagella and diverged centroblepharoplast parts. × 800.

Fig. 15. Entire animal with numerous granular spheres in the cytoplasm, probably parasites. Shows finely alveolar cytoplasm as seen in fixed material. × 410.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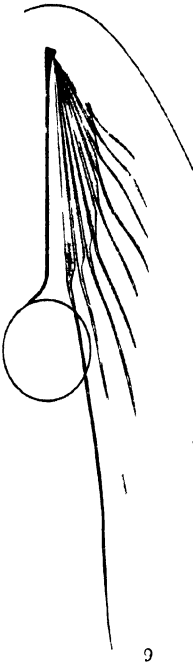
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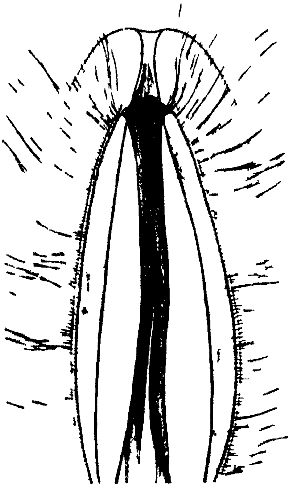
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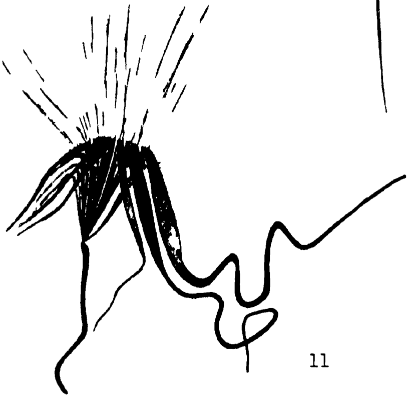
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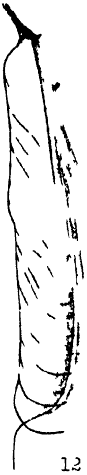
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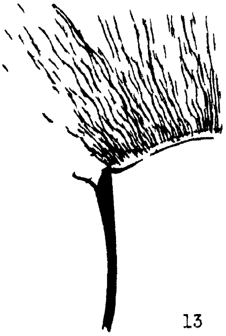
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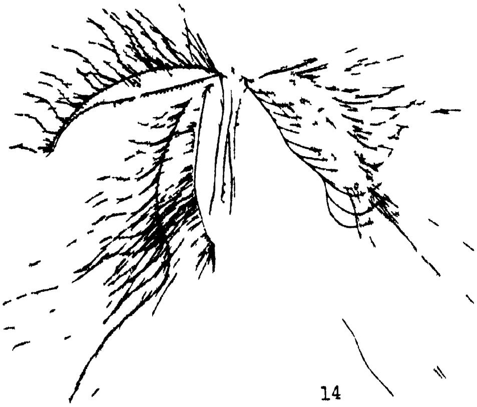
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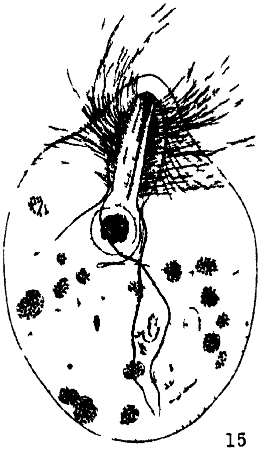
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PLATE 3

Nuclear organization in prophase. × 1500.

Fig. 16. Resting nucleus with clumped chromatin, coarse alveolar zone, and fine alveolar zone.

Figs. 17–19. Resting nuclei with granular central chromatin mass.

Fig. 20. A small nucleus. Chromatin mass breaking up into more distinct granules, preparatory to spireme organization.

Fgs. 21–23. Granules organizing into threads.

Figs. 24–25. Thick, deeply staining threads, numbering 24. A granular area is appearing between the rhizoplast bands where the paradesmose is organizing.

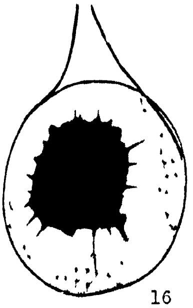
Figs. 26–27. The chromosomes are dispersing in the nuclear space.

Fig. 28. Longitudinal splitting of the chromosomes. Each of the 24 shows a split.

Fig. 29. Split chromosomes, arranged parallel.

Fig. 30. Fibrillar paradesmose appearing between the rhizoplast bands.

Fig. 31. Each of split chromosomes apparently consists of a string of chromomeres.



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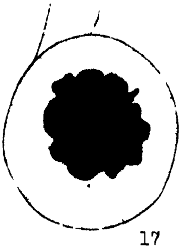

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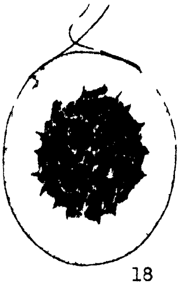

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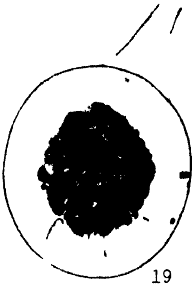

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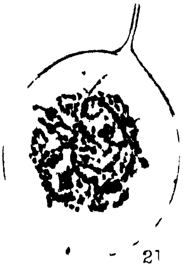

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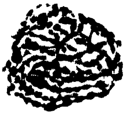

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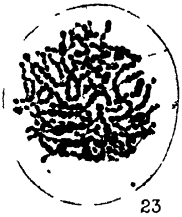

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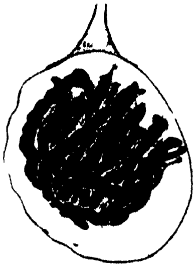

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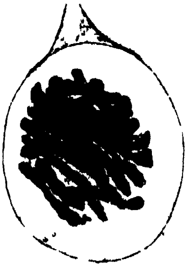

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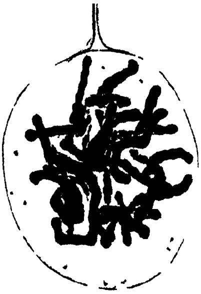

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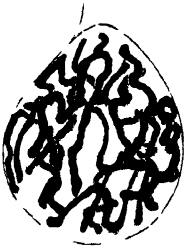

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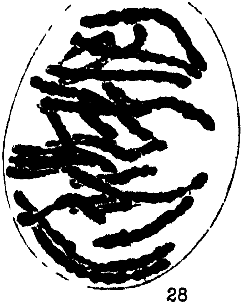

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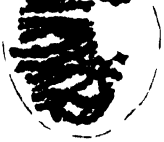

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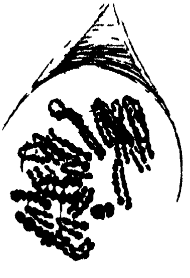

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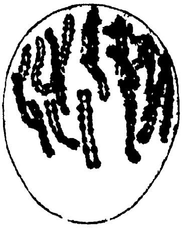

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PLATE 4

Mitotic phenomena. × 1500.

Fig. 32. Split chromosomes shortening.

Figs. 33–35. Shortened chromosomes, still showing split. Fibrillar paradesmose formed in figure 35, astral rays appearing in the cytoplasm.

Fig. 36. Paradesmose sinking in a depression of the nucleus, astral rays radiating from the poles.

Fig. 37. Similar to above save that the chromosomes are elongated.

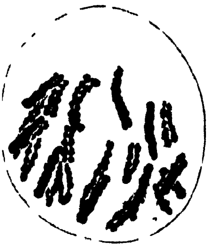
Fig. 38. The arms of the chromosomes are pulling out in V's.

Fig. 39. Number of chromosomes reduced. The separated arms are drawing to the poles, to which they seem to be attached by fibers.

Fig. 40. Chromosomes separated. At right, a section of a flagellar bundle.

Fig. 41. An anaphase in which the club-shaped chromosomes all converge to the poles, from which many astral rays radiate into the cytoplasm.

Fig. 42. Anaphase, showing the large paradesmose and astral rays.



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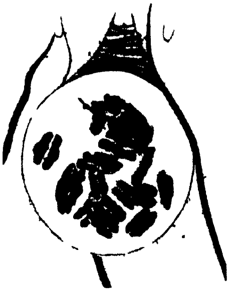

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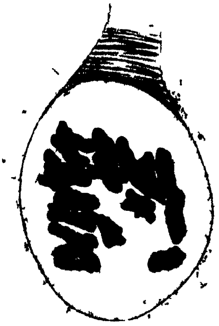

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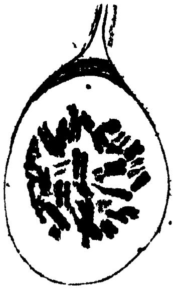

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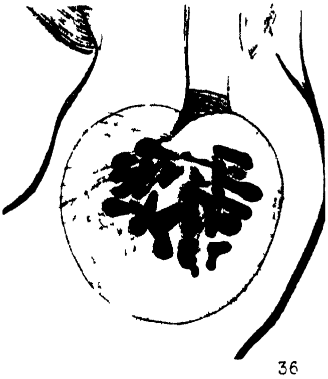

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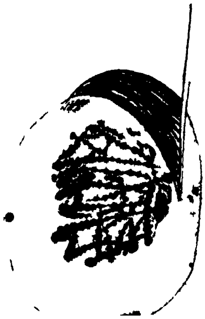

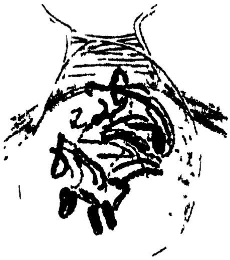

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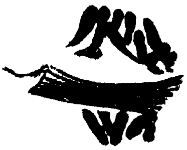

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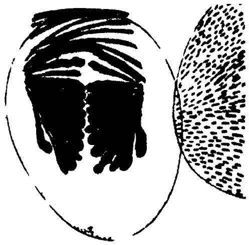

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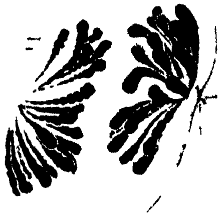

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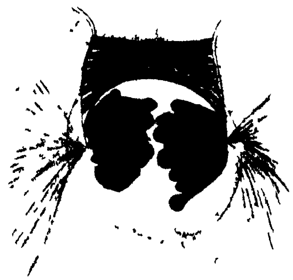

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PLATE 5

Later stages in mitosis. × 1500.

Fig. 43. Anaphase, with beaded chromosomes converging to poles, astral rays radiating into cytoplasm, large paradesmose, and attached rhizoplast bands.

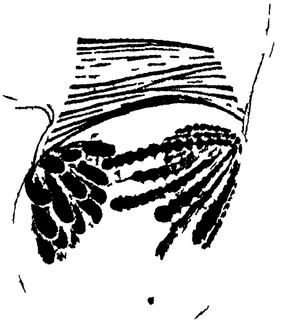
Fig. 44. Later anaphase, as above.

Fig. 45. An end view of nucleus, showing paradesmose sunk in a depression of the nucleus.

Fig. 46. Later anaphase, showing broad rhizoplast bands, stout astral rays, large paradesmose, collection of granules at waist of nucleus.

Fig. 47. Constriction proceeding. At waist of nucleus is some lagging chromatin material.

Fig. 48. Constriction almost complete. Rhizoplast bands broadened.



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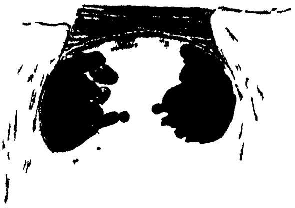

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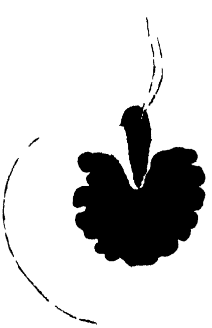

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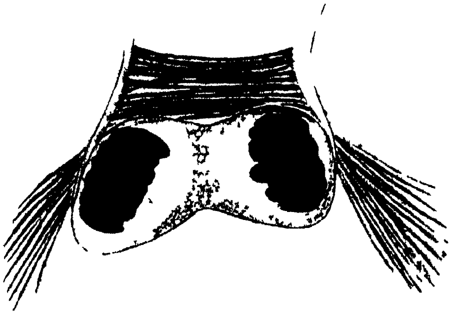

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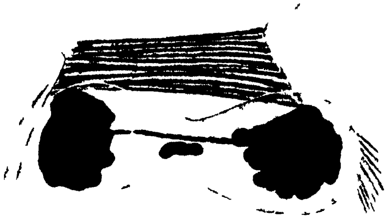

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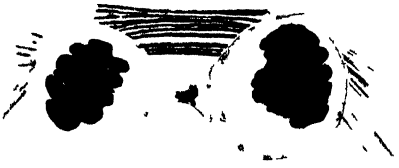

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PLATE 6

Late stages of division and unfixed nuclei. × 1500 unless otherwise stated.

Fig. 49. Nuclei separated. Rhizoplast broadened, the other centroblepharoplast bands broad and associated at ends with paradesmose.

Fig. 50. Paradesmose much elongated and narrowed preparatory to dividing in middle.

Fig. 51. Telophase nucleus, with disintegrating paradesmose and broadened rhizoplast and centroblepharoplast bands.

Fig. 52. Telophase nucleus, showing some lagging chromatin material, disappearing astral rays, converging chromosomes.

Fig. 53. Telophase nucleus in living animal.

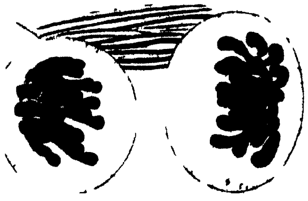
Fig. 54. Paradesmose and broad prophase chromosomes in nucleus of living animal.

Fig. 55. Prophase nucleus of living animal with tangled chromosome threads.

Fig. 56. As above, chromosomes with more uneven margins.

Fig. 57. Two flagellates connected by a long strand. × 215.

Fig. 58. A late stage in plasmotomy. × 215.



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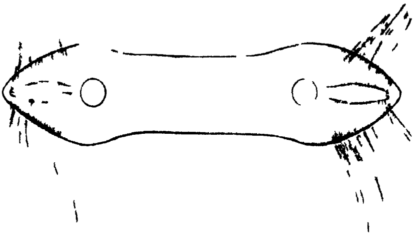

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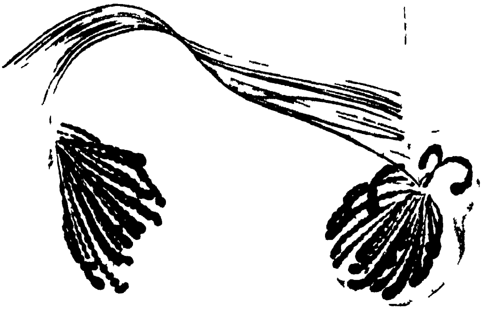

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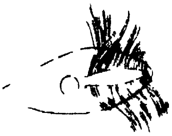

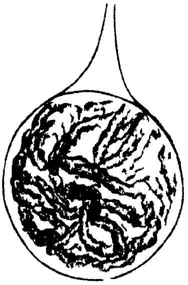

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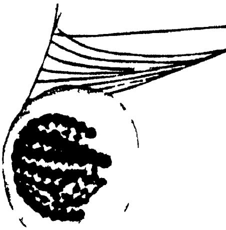

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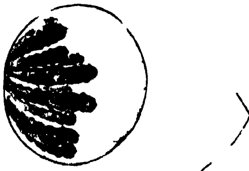

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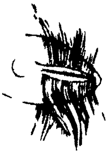

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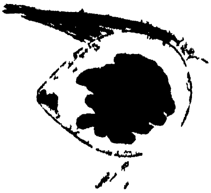

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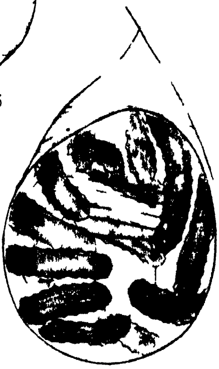

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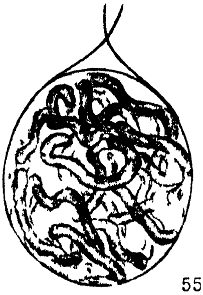

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PLATE 7

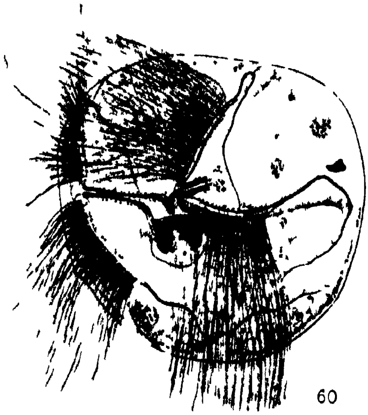
Fig. 59. Whole animal in anaphase. Large paradesmose, astral rays, neuromotor complex divided into two portions. × 410.

Fig. 60. A little later anaphase seen from above. × 410.

Fig. 61. Whole animal in late anaphase. × 410.

Fig. 62. Showing expanding bands which form the new flagellar plates, and the growth of a bundle of flagella. × 800.

Fig. 63. Later stages in growth of flagella. × 800.



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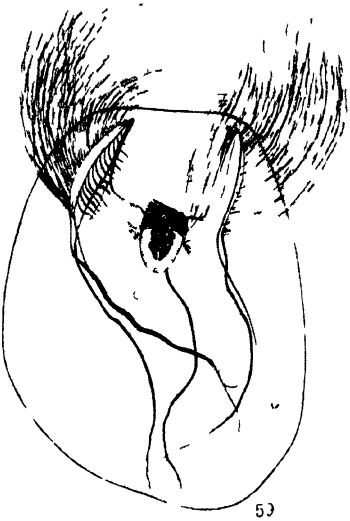

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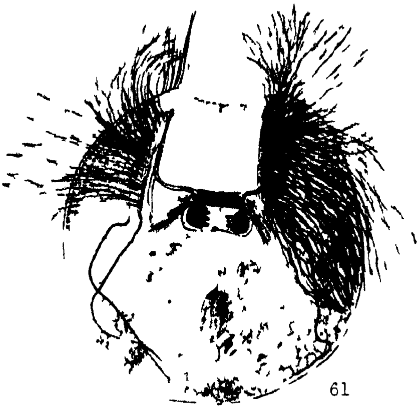

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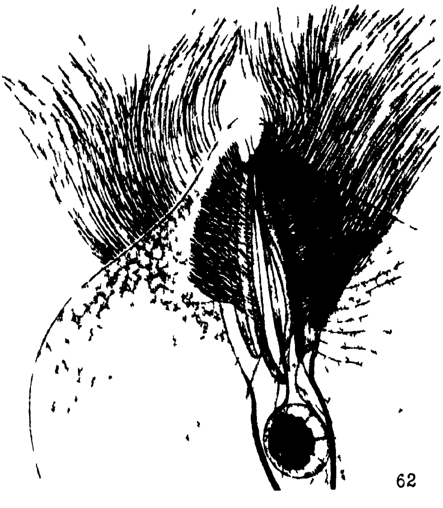

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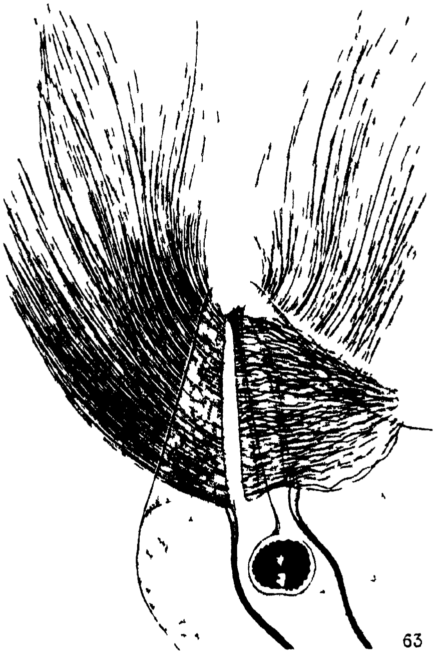

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THE INTESTINAL FLAGELLATES OF THE TERMITE, CRYPTOTERMES HERMSI KIRBY

BY

HAROLD KIRBY, Jr.

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THE INTESTINAL FLAGELLATES OF THE TERMITE, CRYPTOTERMES HERMSI KIRBY

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The material for these studies was obtained at Fanning Island, Central Pacific, in the summer of 1924, and observations on living material were made there at the time. The digestive tracts of the termites were pulled out and fixed in Schaudinn's fluid for periods varying from twenty minutes to two hours, or in Bouin's fixative for several hours. They were preserved in 70 per cent alcohol and subsequently stained in Heidenhein's iron alum haematoxylin, Delafield's haematoxylin or safranin-light green. Some of the haematoxylin stains were counterstained in eosin, a combination which gave very satisfactory results.

I. **Devescovina lemniscata** sp. nov.

The genus *Devescovina* was established by Foa (1905) with the type species *D. striata* from the intestine of an undetermined termite from Chile. Janicki made further studies of this material in 1915, referring the termite to *Calotermes* (*Cryptotermes*) *grassii* Silv. He gave only a brief note concerning *D. striata,* but described fully *Devescovina striata* A. Foa var. *hawaiensis* Janicki from "*Calotermes castaneus* Burm. (Honolulu)." The name of the termite is incorrect. The termite from Hawaii referred to is, according to Snyder (1922), *Neotermes connexus* Snyder. The next reference to Protozoa of this genus is in Grassi's paper (1917), in which he described three new species of "*De Vescovina.*" Franca (1918) described *Caduceia theobromae* from *Neotermes gestri* Silv., a termite from the island of St. Thomas. This may be a *Devescovina.* Exclusive of it, the genus stands now as follows:

Species	Investigator	Host	Locality
D. striata	A. Foa, 1905 Janicki, 1915	*Cryptotermes grassii* Silv.	Iquique (Chile)
D. striata var. *hawaiensis*	Janicki, 1915	*Neotermes connexus* Snyder	Hawaii
D. glabra	Grassi, 1917	*Cryptotermes havilandi* Sj.	Nigeria
D. stereociliata	Grassi, 1917	*Glyptotermes parvulus* Sj.	Gold Coast
D. nova	Grassi, 1917	*Neotermes erytraeus* Silv.	Eritraea

The terms used in the following descriptions are translated from those of the original authors and are not revised.

Devescovina striata Foa

Figure A, 1

Dr. Anna Foa described this very briefly and gave one sketch with some details of structure. The genus was named in honor of Professor De Vescovi, who had supplied the material. The length averages 40 microns, the width 20 microns. On the external surface are fine, discontinuous, longitudinal striations. An internal axostyle extends to within a short distance of the anterior extremity. A parabasal filament begins near the tip of the body, runs past the anterior

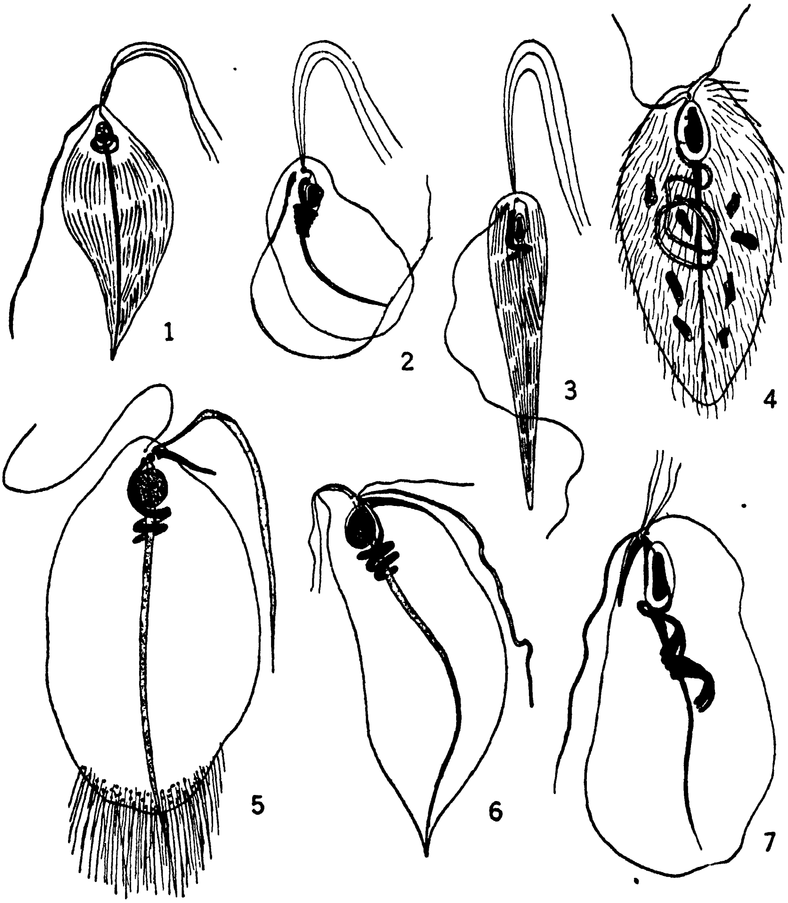

Fig. A. Diagrammatic figures of the described species of the genus *Devescovina*, all × 800. 1. *D. striata* Foa. After Foa, 1905. 2. *D. striata* var. *hawaiensis* Janicki. Broad form. Cuticular striations omitted. After Janicki, 1915. 3. *D. striata* var. *hawaiensis* Janicki. Slender form. After Janicki, 1915. 4. *Caduceia theobromae* Franca. After Franca, 1918. Size computed. 5. *D. stereociliata* Grassi. Size computed, see text. After Grassi, 1917. 6. *D. glabra* Grassi. After Grassi, 1917. *D. nova* Grassi. After Grassi, 1917.

nucleus, and takes two turns around the axostyle. There are three fine anterior flagella and a long membranelle, flattened like a ribbon, which moves like a whip.

Devescovina striata var. **hawaiensis** Janicki

Figure A, 2, 3

Janicki's description is complete, accurate, and well illustrated with 17 figures. The average length is 46 microns, the maximum 55. The width in slender forms is about 10, in broader forms about 25 microns. On the external surface are fine, longitudinal striations, which consist of groups of rods. There is an internal axostyle in the anterior part of which the nucleus seems to be imbedded. The parabasal body takes 1–2 turns about the axostyle in the slender forms, 3–8 in the stouter forms. The three fine anterior flagella are attached to a large, granular blepharoplast; the trailing flagellum is long, flattened, and near its point of origin there is a cuticular structure called *"Schleppgeisselscheide,"* sheath of the trailing flagellum.

Devescovina stereociliata Grassi

Figure A, 5

Grassi's description is very inadequate and his five figures are incomplete. The body is oval, with indistinct traces of transverse striae. A spiral filament is turned about the axostyle back of the nucleus, two turns in the figures, and is prolonged anteriorly. There is at least one delicate anterior flagellum, a long, stout band-flagellum, and a terminal tuft of immobile filaments, which are doubtless bacteria. Possibly related to the band-flagellum is a long, narrow, curved lamella, the "crest," which is separable from the body at the distal end. The size is not given. The size of the diagram, length 60 microns, was computed from the measurements of Grassi's figures of the anterior portion of the body.

Devescovina glabra Grassi

Figure A, 6

Grassi's description of this is brief, but illustrated with some fairly good figures. It resembles *D. stereociliata.* There is an anterior papilla terminating in at least two flagella. The long, flattened trailing flagellum is related to a subtriangular crest, which seems never to project from the body. In the figures this flagellum is not as broad a band as that of *D. stereociliata.* The parabasal body takes two, in one figure three, turns about the axostyle. The size is not given, but one figure (Grassi, pl. 9, fig. 27) is of an animal measuring 57 × 22 microns.

Devescovina nova Grassi

Figure A, 7

A papilla is present, and four (?) delicate anterior flagella. The trailing flagellum is stout but is not flattened. The usual crest is present, and the figures indicate its size as close to that of Grassi's other species, which is greater than that of *D. striata* var. *hawaiensis.* The parabasal body is shown in the figures as large, long, extended some distance into the body, and separated distally into several strands. An individual figured by Grassi (his pl. 9, fig. 28) measures 58 × 27 microns.

Caduceia theobromae Franca

Figure A, 5

Koidzumi (1921) and Cleveland (1923) have said that this is probably a *Devescovina.* A figure is given for comparative purposes.

Devescovina lemniscata sp. nov.

Plate 8, figs. 1–11

D. lemniscata is an abundant flagellate in the intestine of *Cryptotermes hermsi* Kirby. In the living condition the flagellate is oval or pyriform, the slender axostyle projects posteriorly, and it is very active. When degeneration begins the body becomes more elongate. The anterior flagella are organized into a whip which lashes with jerky movements as does the whip of *Pentatrichomonas.* The anterior extremity is prolonged into a snout which terminates in the flagella, and when the animal is fairly quiet the anterior portion of the body may be seen to move with the lashings of the whip. The slender axostyle may project half the length of the body from the posterior end. It may be traced through the cytoplasm to the anterior end, where the oval nucleus lies close beside it. The endoplasm contains granules and, posterior to the nucleus, particles of wood of various sizes, some very small, others about as long as the body.

In fixed material the organism is bluntly narrowed anteriorly, attenuated to the slender, usually projecting axostyle. The length ranges from 12 to 41 microns, averaging 28 microns; the width from 7 to 15 microns, averaging 11 microns. In preservations there are two forms, between which there are intergradations. One is gradually attenuated to a point posteriorly, averaging in size 28 × 11 microns (pl. 8, fig. 1); the other has a rounded body averaging 21 × 12 microns, and a slender axostyle projecting posteriorly for about 12 microns to 22 microns (pl. 8, fig. 9). Some are stouter and larger than usual and possess larger nuclei. Probably these are antecedent to division, as in the case of the larger form of *D. striata* var. *hawaiensis* Janicki. There are discontinuous, rod-like striations on the body surface, as in *D. striata* Foa and *D. striata* var. *hawaiensis* Janicki.

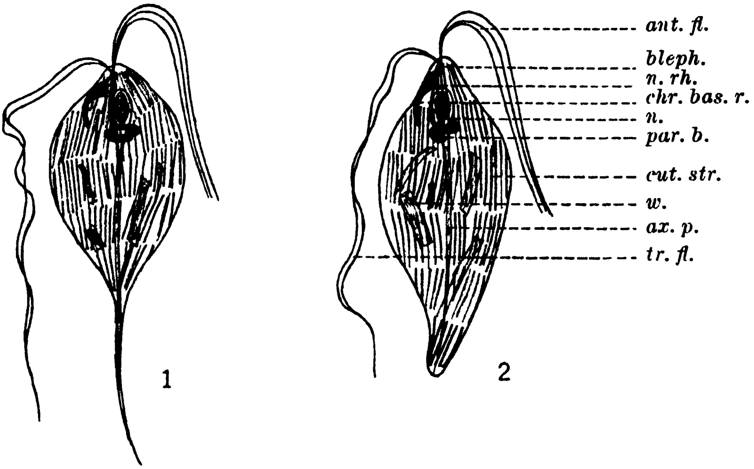

Fig. B. Diagrammatic figures of *Devescovina lemniscata* Kirby. × 1500. 1. Smaller form with projecting axostyle. 2. Broad form with included axostyle.

Abbreviations: As in fig. C. Also *cut. str.*, cuticular striations; *w.*, particle of wood.

In some specimens there is a broad, flattened, pointed projection of the anterior portion of the body on the side opposite to the chromatic basal rod (pl. 8, figs. 2, 3). This is not a usual feature, but is evidently a result of the great mobility of the anterior end. A "papilla" is represented by Grassi in *D. glabra*, and is regarded as an important character of the flagellate; but it is only an occasional feature on stained slides of *Devescovina lemniscata*. That this continues in flagella is certainly not so, though the flagella may lie against and follow the course of the papilla. "Lobopodia" may appear in *Joenia annectens* (Grassi and Foa, 1904) and "papillae" in *Macrotrichomonas pulchra* (Grassi, 1917). It is noteworthy that these structures appear in the region of the cytostome of some Tetramitidae, as in *Gigantomonas herculea* (Dogiel, 1916), *Trichomonas macrostoma* (Dogiel, 1916) and *Trichomitus termitidis* (Kofoid and Swezy, 1919).

The nucleus is anterior and connected to it is a well organized neuromotor apparatus (fig. C) consisting of anterior flagella, trailing band-flagellum, blepharoplast, rhizoplast connecting this to the centrosome on the nuclear membrane, axostyle, parabasal body, and the peculiar "sheath," which has been named the chromatic basal rod.

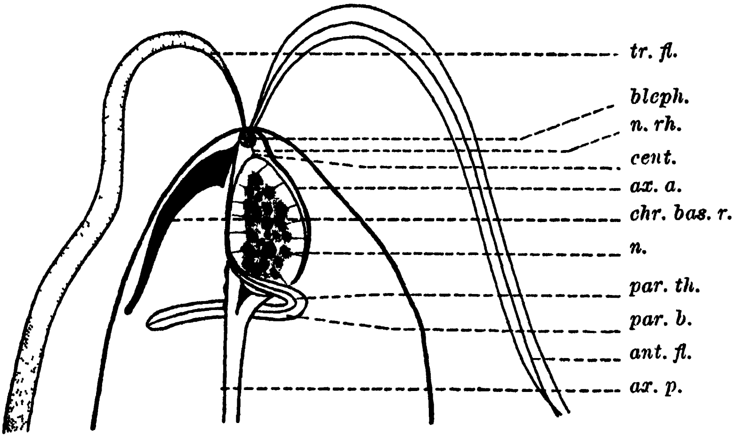

Fig. C. Diagram of the neuromotor system of *Devescovina lemniscata* Kirby. × 3000.

Abbreviations: *ant. fl.*, anterior flagella; *ax. a.*, anterior portion of axostyle; *ax. p.*, posterior portion of axostyle; *bleph.*, blepharoplast; *cent.*, centrosome; *chr. bas. r.*, chromatic basal rod; *n.*, nucleus; *n. rh.*, nuclear rhizoplast; *par. b.*, parabasal body; *par. th.*, parabasal thread; *tr. fl.*, training flagellum.

The nucleus is oval or bluntly narrowed anteriorly, and is surrounded by a distinct nuclear membrane, from which the chromatin material is often contracted so that a clear zone intervenes. In this central mass the chromatin granules are imbedded in a less heavily staining substance. The enlarged nuclei are filled with chromatin granules of variable size and shape.

The blepharoplast is a large body lying close to the anterior extremity. It seems to be composed of a few granules imbedded in a stainable matrix and enclosed within a membrane. To the blepharoplast are attached three long delicate flagella, the ribbon-like trailing flagellum, the parabasal body, and the chromatic basal rod.

The trailing flagellum is a distinctive feature of this species. It is much broader than in any other described species, except possibly *Devescovina stereociliata* Grassi, and it seems much longer than Grassi's figures show the flagellum of this to be. It is attached to the blepharoplast as a thread which soon expands to a band and remains of even width until near the distal end, when it attenuates to a whip-cord. The form of the trailing flagellum varies in different species of *Devescovina,* from a cord in *D. nova* to a broad band in this species.

The sheath, a deeply staining structure at the base of the flagella, is situated just under the body surface and is relatively much larger in this than in the other described species of *Devescovina.* It is subtriangular, blunt distally, attached proximally by a thread to the blepharoplast, and curved around the contour of the body. Where it meets the blepharoplast it is very closely related to the parabasal body. Janicki (1915) regards it as a sheath for the trailing flagellum, and Grassi (1917) seems to have a similar opinion. It is doubtful if it is ever normally free from the body. Doflein states that it is a stereoplasmic structure for anchoring the large flagellum. The function must be the same as that of the chromatic basal rod of the undulating membrane. When safranin-light green is used, this structure stains green.

The parabasal body arises from the blepharoplast as a filament, passes along one side of the nucleus, the side where the chromatic basal rod is situated, and gradually enlarges until when it turns laterally just back of the nucleus it is stout. A parabasal thread runs through its entire length. It takes one complete turn, more rarely two turns, around the axostyle and ends bluntly at its maximum thickness. It never has the large number of coils described in *D. striata* var. *hawaiensis,* nor is it of equal thickness throughout its length as in that flagellate. The parabasal body stains readily with iron haematoxylin and becomes green when safranin-light green is used, when the nucleus becomes red.

The term "parabasal apparatus" was first used by Janicki for the structure in *Joenia annectens* described by Grassi (1893–94) as the *"Collare"* (collar). A similar structure was found in *Lophomonas blattarum* by Bütschli (1878) and Janicki (1910). The parabasal apparatus comprises a parabasal body, which differs morphologically in the different species of parasitic flagellates in which it occurs, and a parabasal thread joining the blepharoplast and often running through the parabasal body. The structure was first named in the Hypermastigina, but a similar body has been found in some of the Tetramitidae. Studies of its occurrence in this family have been made by Janicki (1911, 1915), Cutler (1919), and others. In *Trichomonas* (Janicki, 1911, 1915), *Ditrichomonas* (Cutler, 1919), and *Foaina* (Janicki, 1915), the parabasal body is a club-shaped structure, double in *Foaina,* attached to the blepharoplast, and placed close to the nucleus. In *Stephanonympha* and *Calonympha* (Janicki, 1915; Grassi, 1917) it is similar. In *Devescovina* a club-shaped body is coiled around the axostyle one to seven times, and when it uncoils on the approach of division it is not unlike the club-shaped body of some trichomonads; this body is called the parabasal by Janicki (1915).

In the trichomonads there is a deeply staining structure at the base of the undulating membrane called properly the chromatic basal rod, also chromatic basis, *Basalfibrille, Costa, cote.* Kofoid and Swezy (1915) have regarded this as "homologous with the stout, deeply-staining parabasal which Janicki (1911) described in certain flagellates," and have interpreted the club-shaped bodies described in trichomonads (Janicki, Alexieff, Kuczynski) as the initial stages of new, outgrowing parabasals or chromatic basal rods. However this may be, the two organelles are certainly separate structures in *Devescovina* and in *Paradescovina* (see below), and in our opinion the coiled body is homologous with the parabasal of other authors than Kofoid and Swezy in *Trichomonas.* The "*Schleppgeisselscheide,*" the structure at the base of the trailing flagellum, is probably the homologue of the chromatic basal rod of the undulating membrane.

It may be suggested that the band-flagellum shows the course of evolution of the undulating membrane. It may become more closely related to the chromatic basal rod and eventually attached to the body throughout its length, the "sheath" then increasing in length to become a chromatic basal rod as long as the body. In a species of *Devescovina* from *Kalotermes minor* it is half as long as the body; in *Paradevescovina nana* nearly as long.

The stout, hyaline axostyle may be observed in living animals, when it appears to be a fairly rigid structure. It expands abruptly just back of the nucleus, touching this, and in the core at this point is a deeply staining cone. The anterior portion, as in *Trichomonas augusta* (Kofoid and Swezy, 1915), is very much expanded, nearly as wide as the nucleus, and is narrowed at the end where it touches the blepharoplast. It lies close against one side of the nucleus, even seems to be curved to fit the nucleus, which thus lies in it as in a spoon. The axostyle is enclosed by a sheath, which may be readily demonstrated in some preparations. The "lamella" observed by Janicki is present in the same form and position in this flagellate. He regarded it as a separate structure, functioning as a suspensorial apparatus, but my observations suggest that it is more probably the edge of the proximal portion of the axostyle.

A clear area sometimes apparent near the anterior end is probably a cytostome. As in *Trichomonas augusta* (Kofoid and Swezy, 1915) it is "a broadly comma shaped, hyaline area at the anterior end on the 'ventral' side opposite the undulating membrane," that is, opposite the chromatic basal rod and usual position of the band flagellum.

Division stages were scarce in the material at hand. The few stages seen indicate that the process in *Devescovina lemniscata* is essentially the same as that described by Janicki (1915) in *D. striata* var. *hawaiensis* (pl. 8, figs. 10, 11).

This species is distinguished from others in the following characters:

1. There is a large, subtriangular chromatic basal rod which does not become free distally.
2. The parabasal body is gradually narrowed to its anterior attachment and takes only one, rarely two, turns around the axostyle.
3. There is a large, flattened ribbon-like trailing flagellum.
4. There are discontinuous, rod-like cuticular striations.
5. The size is less than that of the other species described.

Diagnosis.—Sizes average 28 × 11 microns in material of three smears. Body oval or pyriform, frequently with projecting axostyle. The rostrum continues in a whip of three delicate flagella. The neuromotor system comprises these three fine flagella, a band-like trailing flagellum, a blepharoplast, rhizoplast, centrosome, parabasal body, chromatic basal rod, and axostyle. The band-flagellum is relatively broad. The parabasal body is narrowed proximally, broad at distal end, and takes one, rarely two turns around the axostyle. A parabasal thread runs through it. The chromatic basal rod is relatively broad and large. The surface is sculptured with numerous rod-like, discontinuous striations.

Host.—*Cryptotermes hermsi* Kirby, from Fanning Island, Central Pacific Ocean.

II. **Stephanonympha nelumbium** sp. nov.

Plate 9, figs. 12–18

The genus *Stephanonympha* was established by Janicki (1915) for the species *S. silvestrii* from "*Calotermes castaneus* Burm." = *Neotermes connexus* Snyder. In 1917 Grassi added two varieties, *S. silvestrii* var. *neotermitis crythraei* and *S. silvestrii* var. *cryptotermitis havilandi,* from the termites indicated by the variety names. A new species was found abundantly in the intestines of *Cryptotermes hermsi* Kirby.

Janicki characterizes the flagellates of this genus as large, and furnished with a large number of nuclei which are imbedded at the anterior end concentrically or in spiral lines, each nucleus associated with a group of flagella, an axial filament, a parabasal apparatus, and a blepharoplast to which these structures are connected. The axial filaments are collected into a stout bundle, which extends axially through the body.

S. silvestrii Janicki occurs in two forms, f. *minor* and f. *major.* The former is about 69 microns long, the latter about 136. The smaller form possesses about 100 nuclei in 2–4, usually 3, circular series; the larger form about 150 nuclei. These nuclei are spindle-formed, and the dense chromatin is separated by a clear space from the nuclear membrane. Four flagella leave the blepharoplast. The parabasal body is tongue-formed, may be as large as the nucleus, especially in the posterior series.

Grassi's descriptions and figures are incomplete. *S. silvestrii* var. *cryptotermitis havilandi* has small parabasal bodies the outlines of which may be irregular. A parabasal thread connects to the blepharoplast. The figures show for each mastigont 2–4 flagella with indefinite connections. A figure is given of an animal measuring 65 microns long. The number of nuclei and nuclear series is not given, but one figure (his pl. 10, fig. 37) shows four rows. *S. silvestrii* var. *neotermitis erythraei* is said to have a mass of dense plasma close above the nucleus in place of a definite parabasal body. The size, number of nuclei or flagella is not given in the descriptions or figures. In this species are included as developmental forms some *Oxymonas*-like flagellates which certainly belong to a different genus. It would doubtless be better to separate these species from the type species by different specific names instead of retaining the clumsy quadrinomials given them by Grassi.

S. nelumbium is spherical or ovoidal, frequently pointed posteriorly where the axial bundle may project a short distance. The apical portion is clear and the rest of the cytosome crowded with fragments of wood. At the apex are numerous nuclei arranged in a spiral series, and associated with these are numerous active flagella which are as long as the body. The nuclei and axial filaments, being strongly refractive, are readily visible in living material. Ordinary forward motion involves a rapid turning from right to left, and frequently much débris is dragged along behind. Spirilla may adhere to the body surface, frequently forming a thick tuft at the posterior end.

Stained specimens range from 27 to 60 microns in length, averaging about 45; 19 to 40 microns in greatest diameter, averaging about 27; these measurements made on about 200 individuals. The nucleated portion is generally about 9 microns deep and 19 microns across at the base. The number of nuclei varies from about 40 to more than 100, but the usual number is 80 to 100. These are arranged in 5–7, exceptionally 4 series, with the tendency toward a spiral. The axial filaments converge a short distance back of the nuclear crown to a bundle with truncate end, about 3 microns in diameter. This bundle is rigid and resistent.

The nuclei are oval, 1–2 microns in greatest diameter, and set in series close together. The granular, deeply staining chromatin mass is drawn back from the nuclear membrane. At the posterior end of the mass there is often a granule connected to the main mass. Close to the membrane at the top is a relatively large spherical blepharoplast, on top of which is a conical structure. One flagellum arises from the apex of this, three others in a group more laterally. Close to the nucleus is a spherical, superficially placed parabasal body of diameter less than half the nuclear length, along the lower edge of which runs a parabasal thread which connects to the blepharoplast. On the other side the axial filament arises from the blepharoplast, runs posteriorly against the nuclear membrane, and enters the axial bundle.

Few division stages were seen. Prior to division the chromatin becomes more dispersed and fills up the space within the membrane. The nuclei ultimately separate into two groups, the axial bundle also separating (pl. 9, fig. 17).

Forms are present which possess a minimum of cytoplasm. The rounded, compact head possesses 25 or more nuclei with associated flagella and long axial filaments gathered into a bundle. A membrane covers this bundle, but there is no body plasma. This form, which may be degenerative, has been seen in both living and preserved material.

S. nelumbium is probably the smallest thus far described, being much smaller than Janicki's forms. Grassi's cannot well be compared, for he seems to consider the size unimportant. This species is characterized by the parabasal body, number of nuclear series, and blepharoplast structure.

Diagnosis.—Average size 45 × 27 microns. Spirally arranged nuclei in 4–7, generally 6, series. Parabasal bodies small, spherical, with distinct parabasal thread along the lower edge. Blepharoplasts large, surmounted by a conical structure from which the four flagella arise.

Host.—*Cryptotermes hermsi* Kirby, from Fanning Island, Central Pacific Ocean.

III. **Paradevescovina nana** sp. nov.

Plate 9, figs. 19, 20

This small flagellate, which is abundant in the intestines of the termite, closely resembles *Devescovina,* though very much smaller than any described species of that genus. The body is oval and usually the axostyle projects posteriorly for a distance about the length of the body. From the anterior end to the tip of the axostyle the length is about 20 microns, the body itself measuring about 10 microns long, about 7 microns in diameter. The axostyle is stout, very distinct, and may be easily traced to the blepharoplast, a large chromatic body situated anteriorly. From the blepharoplast arise three delicate anterior flagella about 30 microns long and a stout trailing flagellum about 50 microns long. This trailing flagellum, stout proximally, becomes very fine distally. A chromatic basal rod has origin from the blepharoplast and extends posteriorly at the periphery of the body to near the posterior end. It is the parabasal body which distinguishes this genus from *Devescovina.* While in *Devescovina* the parabasal takes one or more turns around the axostyle back of the nucleus, in this organism it runs past the nucleus and approaches the periphery as does the chromatic basal rod, extending posteriorly for half the body length. The parabasal body stains with iron-haematoxylin similarly to the chromatic basal rod. The axostyle, which is relatively much larger than in *Devescovina,* does not occupy the central axis of the body but approaches one side in such a way that the entire course is somewhat sigmoid under one aspect.

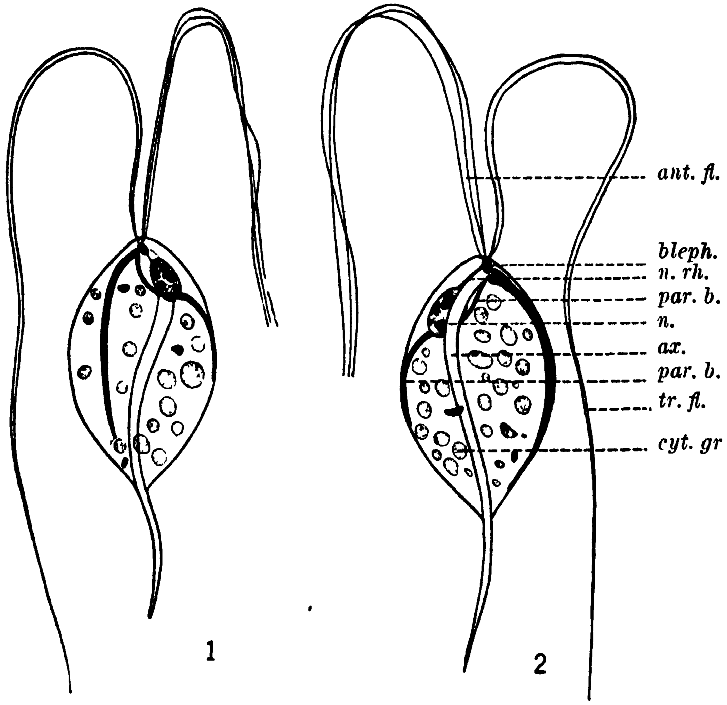

Fig. D. Diagrammatic figures of *Paradevescovina nana* Kirby, × 2500. 1. Parabasal body lying above the nucleus. 2. Parabasal body lying below the nucleus.

Abbreviations: *ant. fl.*, anterior flagella; *ax.*, axostyle; *bleph.*, blepharoplast; *cyt. gr.*, cytoplasmic granules; *n.*, nucleus; *n. rh.*, nuclear rhizoplast; *par. b.*, parabasal body; *tr. fl.*, trailing flagellum.

Paradevescovina gen. nov.

Diagnosis.—Small flagellates with structures similar to those of *Devescovina.* The genus differs from *Devescovina* in that the parabasal body, leaving the blepharoplast and running at first close to the nucleus, turns out to the periphery and continues as a slender rod. The axostyle is stout, curved, or sigmoid in its course.

Type.—*Paradevescovina nana* sp. nov. from *Cryptotermes hermsi* Kirby, Fanning Island.

IV. **Oxymonas parvula** sp. nov.

Plate 9, figs. 21–23

A small flagellate which has been found infrequently on prepared slides corresponds most closely to *Oxymonas gracilis* Kofoid and Swezy (1926) from *Kalotermes* (*Rugitermes*) *magninotus* Emerson from Kartabo. The general form is flask-like or ovoidal with the posterior end truncate, rounded, or pointed. The proboscis may be completely retracted or extended to about the length of the body. Specimens with a range of body length from 5 to 13 microns were measured; in these the longest proboscis measured 10 microns.

Two relatively large blepharoplast granules are located at the base of the proboscis anterior to the nucleus, and from each of these a rhizoplast runs toward the nucleus. These blepharoplasts are connected by a slender circular rhizoplast, as is the case in *O. gracilis*. The axostyle runs from the posterior end of the body to a point anterior to the nucleus and appears to continue in the proboscis as a number of fine fibrils.

From each of the blepharoplasts there extends a group of flagella which exceed the body in length. Though three flagella in each set could be counted only with the greatest difficulty, it is probable that this is the number. Three have been found in other oxymonads by Kofoid and Swezy (1926) and by me in other species in my material.

There are numerous relatively large spherical granules in the endoplasm which do not stain black with haematoxylin. Besides these there are small deeply staining granules and some fragments of wood. Bacteria may adhere in large numbers to the posterior end of the body as shown in plate 9, figure 22.

Due to the very small size of this species the sleeve and retractile fibers described by Kofoid and Swezy could not be observed, though a suggestion of the sleeve was seen in one instance.

This species is smaller than *O. gracilis,* the blepharoplasts are relatively larger, and the flagella seem to be longer. It is scarce in my material, while the other is abundant in its host. The hosts belong to different subgenera and are widely separated geographically. It seems best after consideration of these points to distinguish it by a new specific name as *Oxymonas parvula.*

Oxymonads are widely distributed in termites of the subfamily Kalotermitinae, where they occur in all (23) species which I have examined. They seem to be limited to this group, for they have not been recorded by others nor seen by me in any other termites.

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EXPLANATION OF PLATES

PLATE 8

All figures are of *Devescovina lemniscata* sp. nov. Figures 2, 3, 4, 5, 7, and 8 × 2640; others as stated.

Fig. 1. Broader form showing flagella entire and neuromotor structures. × 1500. Striations not shown. See fig. B.

Fig. 2. Enlarged drawing of anterior end, showing the ''papilla,'' compact chromatin withdrawn from nuclear membrane, parabasal body, chromatic basal rod, blepharoplast, flagella, and branch of axostyle passing over nucleus.

Fig. 3. Similar. Rhizoplast indicated, and chromatic basal rod separated from body, probably abnormally. Nucleus enlarged, in prophase.

Figs. 4, 5. Views of anterior portion of body. In both the rhizoplasts can be seen.

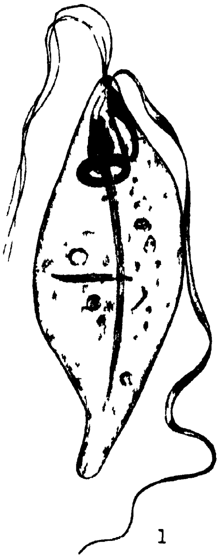
Fig. 6. An individual with lightly stained parabasal body, showing the structure of the anterior part of the axostyle. This expands against the nuclear wall, forming a support for the nucleus. A branch leaves this part of the axostyle and runs over the nucleus toward the blepharoplast. In the center of the axostyle where it touches the nucleus is a deeply staining core. × 1407.

Figs. 7, 8. Additional views of the neuromotor structures. In figure 7 the nucleus is enlarged and the chromatin distributed, preparatory to mitosis.

Fig. 9. A more slender form with projecting axostyle. × 1500.

Fig. 10. A telophase. The paradesmose is V-shaped, connects the two granules at the poles of the nuclei. A large, club-shaped parabasal body is connected to each granule. × 1500.

Fig. 11. An anaphase. A clavate parabasal connects to each granule at the poles of the nucleus, and a paradesmose joins them. × 1500.



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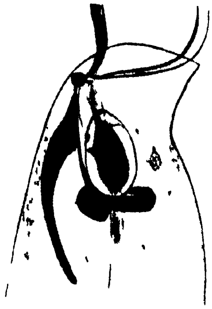

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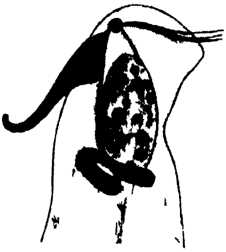

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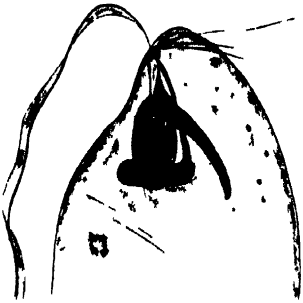

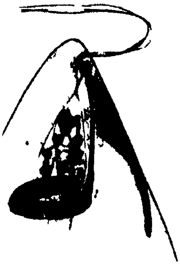

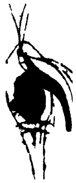

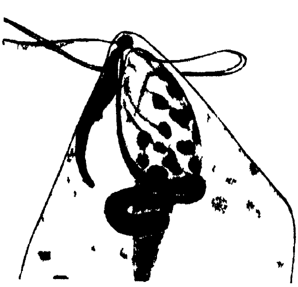

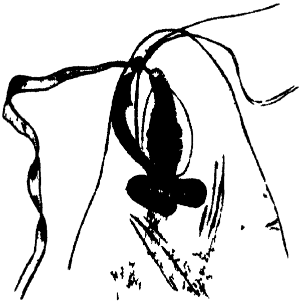

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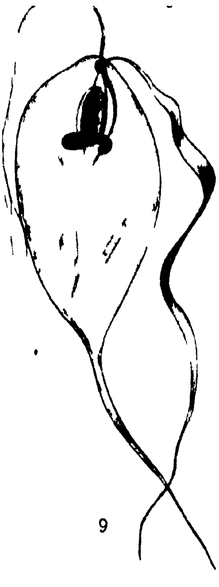

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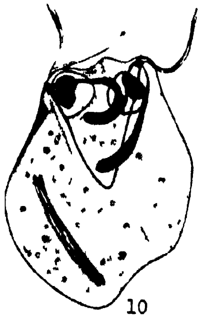

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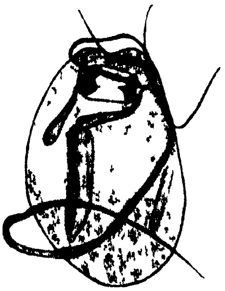

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PLATE 9

Flagellates from *Cryptotermes hermsi* Kirby. × 1500 unless otherwise stated.

Figs. 12–18. *Stephanonympha nelumbium* sp. nov.

Fig. 12. Entire organism, six rows of flagella, bundle of axial filaments. × 800.

Fig. 13. View of the spiral series of nuclei from the posterior end. Four series.

Fig. 14. *a.* A single mastigont, showing nucleus, blepharoplast, flagella, parabasal body, parabasal thread, axial filament. *b.* An enlarged figure of the same.

Fig. 15. *a.* A single mastigont as above. The parabasal thread is against the lower edge of the parabasal body. *b.* An enlarged view of the same.

Fig. 16. An optical section of an individual with six nuclear series. × 800.

Fig. 17. A mitotic figure. The two groups of nuclei and the axial bundles are separated. × 800.

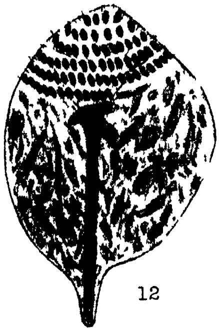
Fig. 18. A few mastigonts, showing the arrangement of nuclei, parabasal bodies and axial filaments.

Figs. 19, 20. *Paradevescovina nana* sp. nov.

Figs. 21–23. *Oxymonas parvula* sp. nov.

Fig. 22. Spirilla adherent to the posterior end of the body.

Fig. 23. Seen from the anterior end, showing the two blepharoplasts and two sets of flagella.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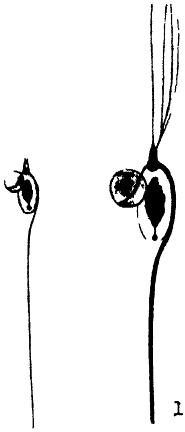
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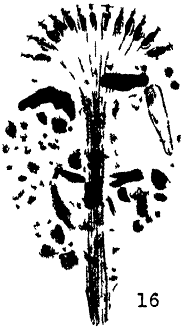
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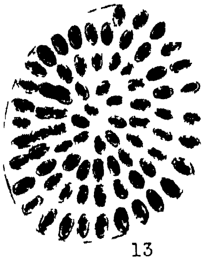
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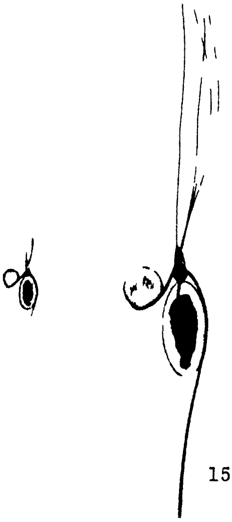
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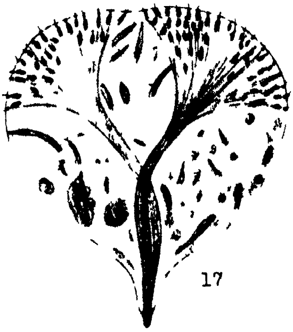
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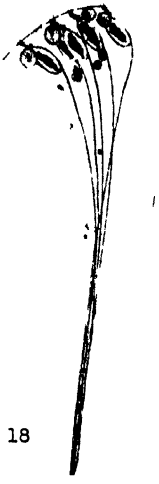
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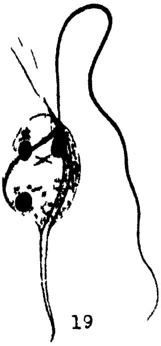
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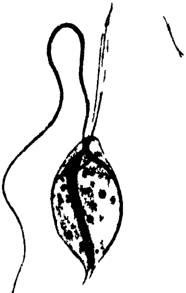
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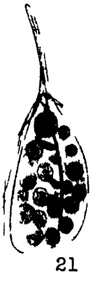
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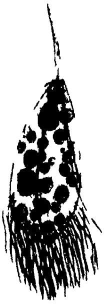
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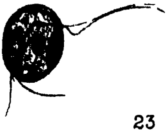
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ON HOPLONYMPHA NATATOR GEN. NOV., SP. NOV.

A NON-XYLOPHAGOUS HYPERMASTIGOTE FROM THE TERMITE, KALOTERMES SIMPLICICORNIS BANKS, CHARACTERIZED BY BIRADIAL SYMMETRY AND A HIGHLY DEVELOPED PELLICLE

BY

S. F. LIGHT

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The intestine of the lower families of the termites has provided sanctuary wherein there have been preserved the most astounding examples of complex flagellate organization. Here we find Protozoa of amazingly complicated and diverse organization swarming over one another in a seething, nearly compact mass of living substance. In the intestine of *Kalotermes simplicicornis* Banks we see this condition at its climax. At least ten species of flagellate protozoans are to be found in abundance in the intestinal contents of every individual of this species. These ten species represent nine genera, distributed among six families belonging to two different orders of flagellates.

On viewing this swarming museum of protozoan marvels one is confronted by numerous questions to whose answers some clues at least may be found in studies of the morphology and biology of these Protozoa. These clues in turn may throw light into some dark corners of our evolutionary thinking and will undoubtedly be of the greatest importance in any study of termite phylogeny. The need at present is for the accumulation of a greater body of data as to these Protozoa. We may, however, receive interesting intimations by the way as to the significance of these data.

With a view to obtaining material for such studies on the Protozoa of American termites the author undertook a collecting trip in southern Arizona in May, 1925. The termites collected have been reported by Dr. T. E. Snyder of the United States Bureau of Entomology (1926). Among them was *Kalotermes simplicicornis* Banks, several colonies of which were found in Copper Butte Cañon near Ray, Arizona. It had previously been taken but twice, once in Nueces County, Texas, and once in Sabino Cañon, near Tucson, Arizona. It has since been sent to us by the Southern Pacific Railway from the Los Angeles Division in this State, and Mr. Lamb, collector for the Museum of Vertebrate Zoology of the University of California, has taken it in Lower California.

With few exceptions, perhaps more properly with none, the species of the termite genus *Kalotermes* (Hagen) Banks are characterized in the soldier caste by the enlarged, darkly colored, and heavily chitinized third antennal segment. This character, as the specific name indicates, is lacking in *K. simplicicornis,* the third antennal segment being of the same relative size, shape, and color as the other segments. As will be brought out more fully in later papers this morphological difference is accompanied by a striking difference in its protozoan fauna as contrasted with the faunas of other species of the genus. So entirely unique is this fauna that, when correlated with the unique morphological character of the antennae of the soldiers, it would seem to justify the prediction that when the adults are known they will present characters which will make it necessary to consider *K. simplicicornis* as representing a generic type distinct from that of the other American species of *Kalotermes.*

Hoplonympha natator gen. nov., sp. nov., described in this paper, is among the smaller forms found in the intestine of *K. simplicicornis,* but its unusual form and striking swimming movements at once attract the attention of the observer. It is a constant element of the intestinal fauna of this species, present in considerable numbers but making up a relatively small percentage of the total fauna, as would be expected in view of the fact that it is not seen with included wood or other food bodies and hence must be in the nature of a saprozoic dependent.

I wish to take this occasion to express my gratitude to Dr. C. A. Kofoid with whom this work was done, for his interest, his constant aid, and his helpful advice. To Mr. Henri Behoteguy, of Phoenix, Arizona, I am much indebted for aid while in the field.

Kirby (1926) has given a very complete bibliography and review of the literature on the Protozoa of the termites. The author will confine his references, therefore, to such papers as are immediately germane to the discussion.

MATERIAL AND METHODS

This study was made on both living and fixed material. The termites were kept in the wood in which collected and transferred to filter paper for some time before being used for study. Examinations of the gut content were made in Locke's solution. No really satisfactory indifferent fluid has yet been devised.

Smears were fixed in Bouin's, Schaudinn's, Zenker's, and Fleming's fluids, and in osmic acid, and stained by the iron haemotoxylin method. Some were prepared by the phosphotungstic acid haematoxylin method and a few stained in Delafield's haematoxylin. Best results for all the flagellates of the termites have been obtained after twenty to thirty minutes in Bouin's fluid at 55° C. For the small forms, including the one described in this paper, a long stain in haematoxylin ripened with lead acetate followed by a counterstain in water soluble (yellow) eosin gave excellent preparations. Light green proved an excellent counterstain for certain purposes. Da Fano's method while distorting the organism brings out the pellicular ridges in splendid relief.

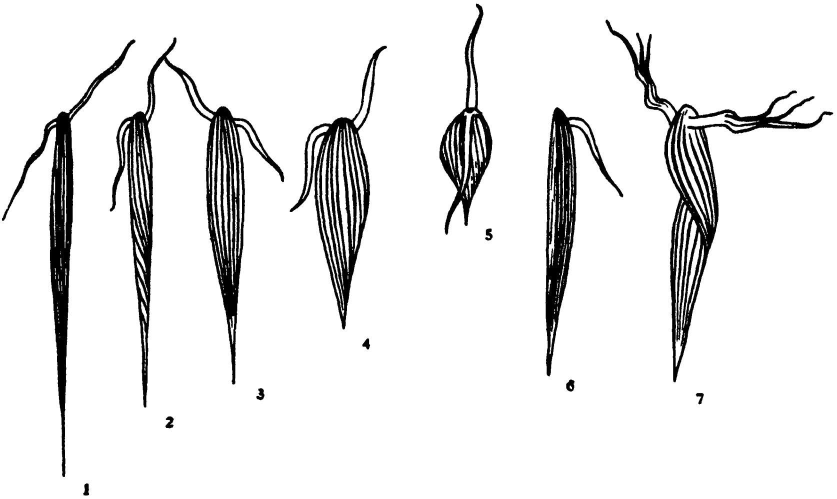
GENERAL DESCRIPTION

SHAPE AND SIZE

Hoplonympha natator is cigar-shaped, long and slender (fig. 10), 90 (60–120) microns in length and 9 (5–12) microns in greatest diameter. The body is stoutest at or somewhat posterior to the level of the nucleus. From this point it tapers somewhat rapidly to the roundly pointed anterior tip and very gradually to the long needle-like posterior tip (figs. 1, 8–11). The short, narrow, roundly pointed region anterior to the posterior margins of the flagellar bundles may be designated as the nipple (fig. 10). There is a considerable range in size and proportions (figs. 1–7), but apparently very little, if any, rapid change in the shape of the individual under normal conditions. In artificial media the body usually becomes much shortened and thickened (figs. 4, 28). At the time of mitosis the body is considerably shortened and thickened, particularly during plasmotomy (fig. 22).

PELLICLE

Perhaps the most striking structural character of *Hoplonympha natator* is the great development of the pellicle which forms a relatively thick and rigid armor over the entire body, hence the generic name. This pellicular armor is thrown up into a series of about 12 to 15 conspicuous, close-set, linear ridges separated by deep, usually narrow, grooves (figs. 10, 12, 16). Originating very near the anterior tip (fig. 10) these ridges take a slightly spiral course running posteriorly from left over to right, i.e., in a weak leiotropic spiral.



Figs. 1–7. Figures from life of *Hoplonympha natator* gen. nov., sp. nov., from *Kalotermes simplicicornis* Banks to show some of the common variations in shape and size. × 500.

1–4. Forms commonly encountered under normal conditions.

5. A greatly shortened type found in starving termites.

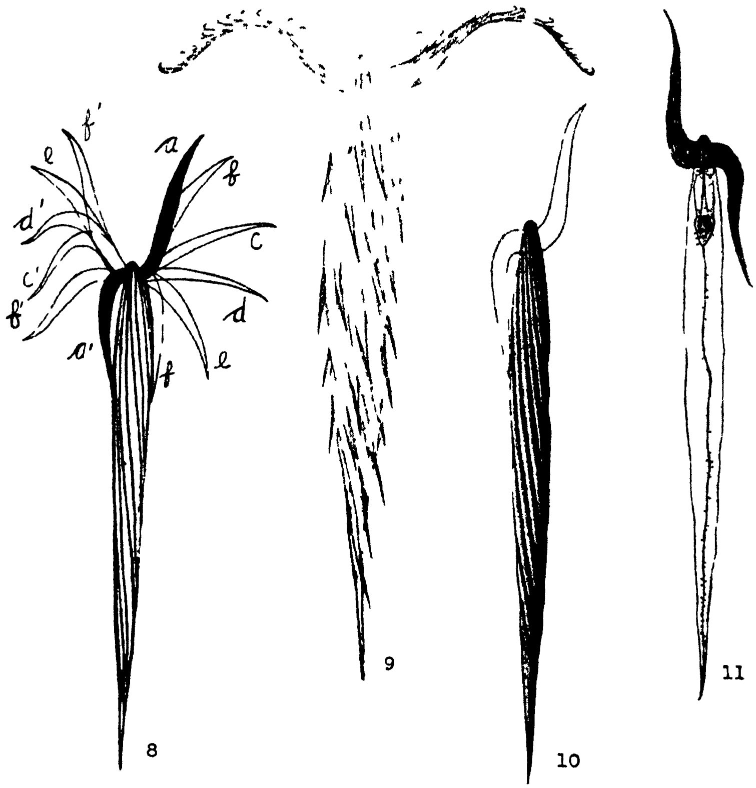
6. An individual with but one flagellar bundle and the opposite side without pellicular ridges. Taken to be a recently divided form.

7. Showing the flattening and twisting of the body and the initial fimbriation of flagellar bundles often seen in individuals recently placed in artificial media.

Material prepared by Da Fano's method is so strongly distorted as commonly to show these ridges in a dexiotropic spiral. This spiral arrangement is not due to a mere temporary torsion but is found in all individuals in more or less marked degree and is still indicated in the earlier stages, at least, of plasmotomy (fig. 22). In fixed material as in life these ridges appear darker along their margins because of the greater depth of pellicle in view at these points and hence they are seen as double lines on the body surface (figs. 14, 23). All of the ridges run to the posterior region of the body but there they end at varying levels and the long slender tip is clothed with an unridged pellicle (fig. 10). The remarkable fimbriation of the pellicle which marks the reaction of *Hoplonympha* to conditions in artificial media is discussed under behavior.

NEUROMOTOR SYSTEM

The neuromotor system of *Hoplonympha natator* is of a type not to be homologized, in its details at least, with that of any known flagellate. The flagella arise in two *flagellar bundles* of about thirty flagella each from opposite sides near the anterior tip, giving the body a distinct bilaterality (figs. 11, 12, 23). Under natural conditions the flagella of each bundle are integrated to form a tentacle-like structure (fig. 10) which acts as a unit and exhibits characteristic swimming beats accompanied by characteristically graceful curves of the flagellar



Figs. 8–11. All figures of *Hoplonympha natator* gen. nov., sp. nov., from *Kalotermes simplicicornis* Banks. 8–10, from life.

8. Diagram showing positions taken by flagellar bundles in complete stroke and return; *a-f*, on right side, various consecutive positions in the effective, or down, stroke; *a'-f'*, on the left, corresponding positions in the upstroke. Bundle in black of each side is in initial position for stroke of that side. × 800.

9. Showing initial stages of pellicular fimbriation and position assumed by flagella when brought to a standstill by abnormal conditions. × 1500.

10. Normal individual represented as opaque to bring out arrangement of pellicular ridges. × 900.

11. Median, longitudinal, horizontal, optical section of a fixed and stained individual similar to the one represented in fig. 10. × 900.

elements during their retarded beat (figs. 7, 8). In artificial media fimbriation soon sets in (figs. 7, 9). In fixed material fimbriation is usually complete (fig. 23). In this condition the flagella are seen to be graduated in length, increasing in length posteriorly. Some of these flagella may be seen to fimbriate at their tips, indicating still smaller fibrillar units (fig. 23). At its origin each bundle is roundly triangular in section with the apex turned anteriorly (figs. 20, 21). Distally the bundle is vertically flattened, giving a greater propelling surface (fig. 7).

Within the nipple is a *central core* (fig. 18) of dense cytoplasm which ordinarily stains lightly. Applied along the margin of this, or continuous with it, are the flagellar plates mentioned above. Running through it or along its surface are the *rhizoplast bands* (*rh. bds.*, fig. 12) described below. The whole cone-shaped mass within the nipple, including flagellar plates, anterior rhizoplast bands, and the central core, is here termed the *anterior cone* (figs. 13–15). Under certain conditions, particularly when the smear has been allowed to dry somewhat before fixation, the entire anterior cone presents an intense homogeneous stain (fig. 17).

Where the two flagellar plates and the rhizoplast bands meet anteriorly a granule or irregular siderophile mass is to be made out in some preparations (figs. 13, 19). As the only visible center of integration, and in the absence of any evidence from early mitotic stages, this has been designated tentatively the *blepharoplast*. When visible it is seen to lie at the center of concavity of a tiny, terminal, bowl-shaped structure, termed here the cap (*cap*, fig. 12; figs. 13, 15, 17, 18, 19, 23). Within the cap chromatic granules are universally present which show a considerable range of variation as to number, size, and arrangement. By far the most common and apparently the typical arrangement is that of a slender funnel of delicate chromatic granules, called here the *chromatic funnel* (*ch. fun.*, fig. 12), with its stem resting on the blepharoplast and ending in a flaring mouth resting broadly against the anterior convex boundary of the cap. This cap with its chromatic elements may be a vesicular blepharoplast. One is led to speculate, however, as to the possibility that it is a delicate sensory organelle containing granules sensitive to changes in pressure or chemical gradient.

Between the nucleus, which lies near the anterior end of the body, and the anterior cone lies a column of differentiated cytoplasm called here the *granular column* (*gran. col.*, fig. 12; figs. 11, 13, 18–21) from the large number of eosinophile granules which are seen to characterize it in certain preparations, particularly in its peripheral and posterior regions. Its central and anterior regions seem often to be composed of material of the same nature as, and continuous with, the central core of the anterior cone (fig. 18). This is particularly true in what has been described later as the premitotic phases. Bounding this column in its lateral regions are bands of large, often somewhat chromatic granules known here as the *chromatic bands* (figs. 12, 13, 15, 18, 19). These vary widely in prominence and may very well be, in part at least, simply condensations of the granules of the column preliminary to mitosis. Anteriorly the chromatic band of each side is interrupted by a clear vesicle, the *lateral vesicle* (*lat. ves.*, fig. 12), containing one to several vaguely siderophile granules (*chr. gr.*, fig. 12). These vesicles vary considerably in size and position and the granules vary as to size, number, and staining reactions (figs. 18, 19, 21). In many individuals there appears a clear area, much shorter than the vesicles under ordinary conditions, extending to the periphery on either side between the chromatic band and the granular column posteriorly and the anterior cone anteriorly. It seems probable that this is a pre-mitotic condition preliminary to the formation of the siderophile plates of the mitotic figure. In some interphase stages the chromatic granules of the vesicles appear continuous with those of the chromatic bands (fig. 15).

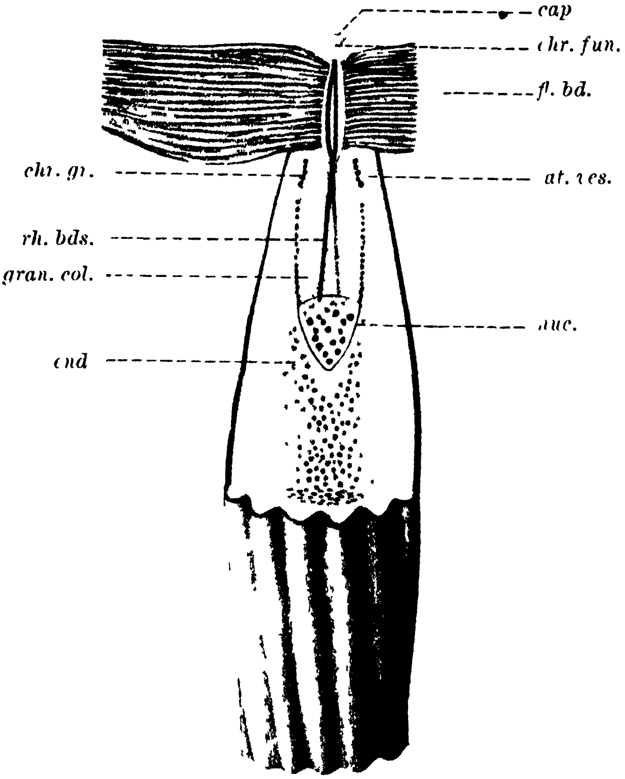
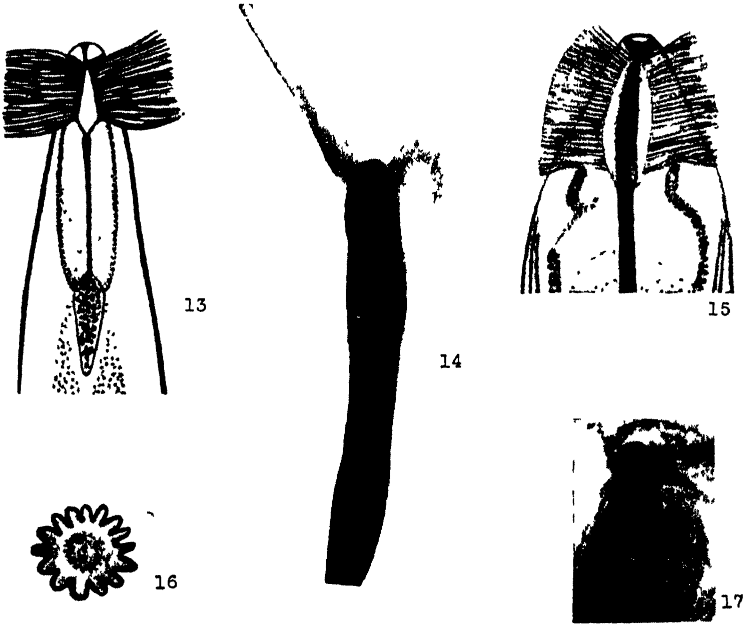


Fig. 12. Enlarged diagrammatic figure of anterior end of *Hoplonympha natator* gen. nov., sp. nov., from *Kalotermes simplicicornis* Banks; anteriorly in mid-longitudinal, horizontal, section and posteriorly in surface view to bring out nature of pellicular armor. × 2500.

Abbreviations: *cap*, cap; *chr. fun.*, chromatic funnel of cap; *chr. gr.*, chromatic granules of lateral vesicle; *end.*, endoplasm filled with large achromatic granules; *fl. bd.*, flagellar bundle, shown as if cut off near point of emergence; *gran. col.*, granular column bounded laterally by chromatic bands; *lat. ves.*, lateral vesicle; *nuc.*, nucleus; *rh. bds.*, rhizoplast bands (anterior end of upper band shown as if tipped to left).

Piercing the center of the granular column is a pair of structures, usually closely associated in the anterior region of the column, where they lie one above another in the vertical plane, which have been designated *rhizoplast bands* (*rh. bds.*, fig. 12; figs. 11, 13, 15, 17, 19), because of their probable homology with somewhat similar structures in *Staurojoenina* (Kirby, 1926). In the anterior region of the granular column the bands are thick, club-shaped, and strongly siderophile. Posteriorly they become attenuated and lose their staining power, their connection to the nucleus being often obscure.



Figs. 13–17. Camera lucida drawings and photomicrographs of *Hoplonympha natator* gen. nov., sp. nov., from *Kalotermes simplicicornis* Banks, from smears stained by the iron haematoxylin method.

13. Anterior end of individual in early premitotic stage. Rhizoplast bands not yet separated posteriorly. × 2700.

14. Photomicrograph of late premitotic stage. On posterior surface note pellicular ridges in characteristic picture as double dark lines. Ca. × 1200.

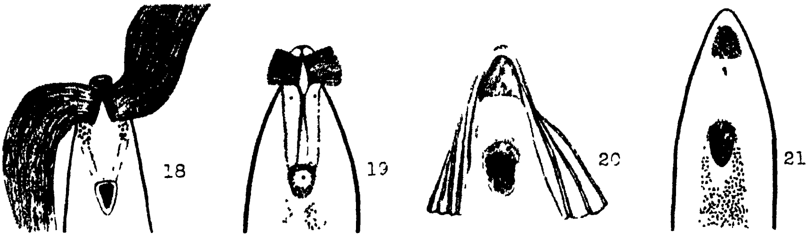
15. Anterior end of individual in interphase. Note apparent continuity of chromatic granules of lateral vesicles and chromatic bands. × 4200.

16. Transverse optical section at level of nucleus, of animal standing on end, to show pellicular armor and thick layer of apparently homogeneous ectoplasm. × 2000.

17. Photomicrograph of flattened individual. Note cap with distorted chromatic funnel, the rhizoplast bands, widely separated because seen from the side and because of flattening, and the deeply stained anterior cone and nucleus. × 1000.

Each is continued, anterior to the column, along the dorsal and ventral margins, respectively, of the central core to the blepharoplast as a more or less siderophile, narrow plate or thread. During the interphase these run normally in the same plane as the posterior portions, i.e., in the vertical plane, and have the appearance, seen from above, of forming a dense, strongly siderophile center to the anterior core (fig. 15).

A considerable number of individuals are seen in which the rhizoplast bands cannot be made out (fig. 18) or are seen to be separating and moving laterally (fig. 19). These individuals are further characterized (1) by the wide separation of the flagellar plates which are now more susceptible to the stain and are broader, resulting in a broader anterior cone and nipple and bringing the posterior ends of the flagellar plates in line with the anterior ends of the chromatic bands (fig. 18); (2) by the greater breadth and prominence of the chromatic band which now seems to include a condensation of the granules from the granular column (fig. 18), giving the central region of the column, particularly in its anterior region, the same appearance as the central core (fig. 18); (3) by a very distinct convergence of the chromatic bands toward the nucleus (figs. 14, 18).



Figs. 18–21. Camera lucida drawings of the anterior end of *Hoplonympha natator* gen. nov., sp. nov., from *Kalotermes simplicicornis* Banks made from smears stained by the iron haematoxylin method. × 1300.

18. Late premitotic stage. Note large lateral vesicles with numerous chromatic granules.

19. Early premitotic stage, rhizoplast bands separating posteriorly.

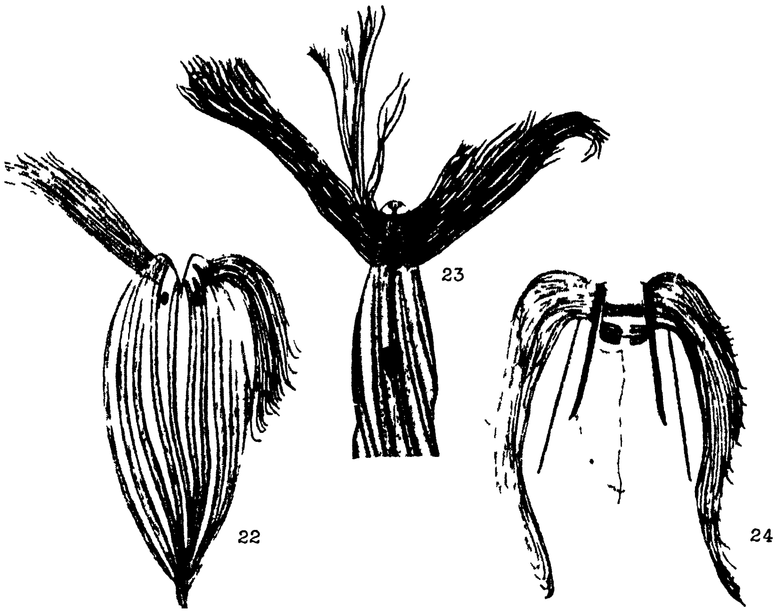
20. Pellicle disrupted in smearing showing actual suspensory nature of granular column. Greater apparent size perhaps due to flattening.

21. Seen from side showing shape of flagellar bundle at base.

It will be noticed that the flagellar plates and the chromatic bands are now in a position suggestive of the origin of the siderophile plate described below under mitosis (cf. figs. 14 and 24). This with the broadening of the body seems to mark this stage as one preliminary to mitosis and hence in spite of certain indications to the contrary it will be tentatively designated the premitotic figure.

That the two pictures described represent stages in the cell cycle seems the more probable from the fact that the individuals of a given smear, i.e., from the same termite, seem nearly always to show the same picture. That this difference in picture is not merely due to treatment is clearly shown by some smears which show both conditions and also stages intermediate between them. These intermediate stages show the separated flagellar plates but the rhizoplast bands while separated or invisible anteriorly are still more or less in normal position within the granular column (figs. 13, 19).

From the posterior margin of the nucleus a slender granular line may be seen in some individuals, particularly those in mitosis, to take a meandering course through the narrow core of granular endoplasm to the extreme posterior tip of the body. This structure designated here the *endoplasmic thread* (figs. 11, 24) may possibly represent a vestigial axostyle.



Figs. 22–24. Camera lucida drawings of *Hoplonympha natator* gen. nov., sp. nov., from *Kalotermes simplicicornis* Banks: 22 and 23, iron haematoxylin; 24, Delafield's haematoxylin.

22. Early stage in plasmotomy. Note greatly reduced siderophile bars and nearly straight pellicular ridges, evidently equally shared between daughter organisms. × 1200.

23. Anterior end of individual in interphase stage. Note fimbriated ends of some flagella. Pellicular ridges represented as double dark lines as they ordinarily appear. × 1700.

24. Anterior end of individual in anaphase stage of mitosis, showing siderophile bars, threads to nucleus (rhizoplast bands?), paradesmose, chromosomes, endoplasmic thread and absence of granulations in region formerly occupied by granular cone. × 1700.

Nucleus

The nucleus has the shape of an inverted cone. It rests with the anterior, broader end embedded shallowly within and suspended by the granular column (*nuc.*, fig. 12). The nuclear contents consist of a few rather large, strongly siderophile granules in an extensive gray, achromatic matrix (figs. 18–21). In certain preparations, particularly those presenting the premitotic picture, the nucleus is narrow, the chromatin is more prominent (fig. 18), and the vesicular structure, characteristic of the nucleus of the hypermastigotes generally, is distinct (fig. 14). In individuals in the interphase the clear marginal zone is not ordinarily to be made out. A spherical siderophile granule in a clear halo, perhaps a karyosome, is to be made out in certain preparations (figs. 19, 21).

CYTOPLASM

The cytoplasm consists of a deep layer of apparently structureless ectoplasm (fig. 12) surrounding the anterior cone and the granular column anteriorly and, posteriorly to the nucleus, surrounding an endoplasmic core characterized by numerous large granules which take none of the stains used (figs. 11, 12, 13, 14, 19, 21). No one of the thousands of individuals examined has been seen to contain food bodies and indeed it is difficult to conceive of their being able to pass the heavy pellicle. The few inclusions seen were of the same general appearance and located in the same region of the body and it seems probable that these are parasites. We are forced to the conclusion, therefore, that we are dealing here with organisms dependent for their food supply on the dissolved substances of the intestinal fluid.

DIVISION

The material for the study of the division processes of this most peculiar flagellate is far from complete but it is sufficient to indicate the unique nature of these processes. The earlier stages have not been seen. In metaphase and later stages the nucleus is seen to have moved anteriorly and to be located at about the level of the posterior ends of the flagellar plates and to lie between two heavy, laterally placed, deeply staining siderophile bars (figs. 22, 24). The flagellar bundles have much the same relation to the anterior portions of these bars as to the flagellar plates of the interphase and it seems legitimate to suppose that this portion of each bar represents the flagellar plate of that side plus, perhaps, a part of the central core. From its position and appearance the posterior portion of each of these bars would seem to represent a condensation of the chromatic band of that side plus the granules of the granular column, for the protoplasm of that region is now seen to be loosely alveolar (fig. 24) and to lack the granules which characterize it during interphase. Further, premitotic figures show an apparent condensation of these granules (figs. 13, 18).

In the one clear anaphase figure available in equatorial view a distinct thread is seen running from the pole of each chromosome group up along the inner surface of the siderophile bar to end without determinable connections at the anterior end (fig. 24). The origin of these threads, like that of the siderophile bars, must remain uncertain in the absence of the early mitotic stages. It seems most likely that they represent the rhizoplast bands which probably function to draw the nucleus forward. Between these threads, which we may tentatively call the rhizoplast bands, and just anterior to the nucleus, is a band (fig. 24) somewhat fibrous in appearance which takes the stain but weakly but nevertheless seems to be in the nature of a *paradesmose.*

The cap with its chromatic funnel is indistinguishable as such at this time, although the fibers at the anterior end of each siderophile bar (fig. 24) would seem to represent the latter. The possibility remains that the chromatic granules of the cap represent a centroblepharoplast and that the threads, i.e. the rhizoplast bands, take their origin in it.

There remain the lateral vesicles with their chromatic granules to be considered. These are always present during interphase with no apparent function. During mitosis they have not been made out as such in the few specimens available. We would seem justified in believing them to be concerned in the mitotic process, but what their function is remains to be discovered.

The chromatin of the dividing nucleus takes a light stain as contrasted to the siderophile bars (fig. 24). The chromosomes are few in number, probably from 4 to 8, but have not been definitely counted.

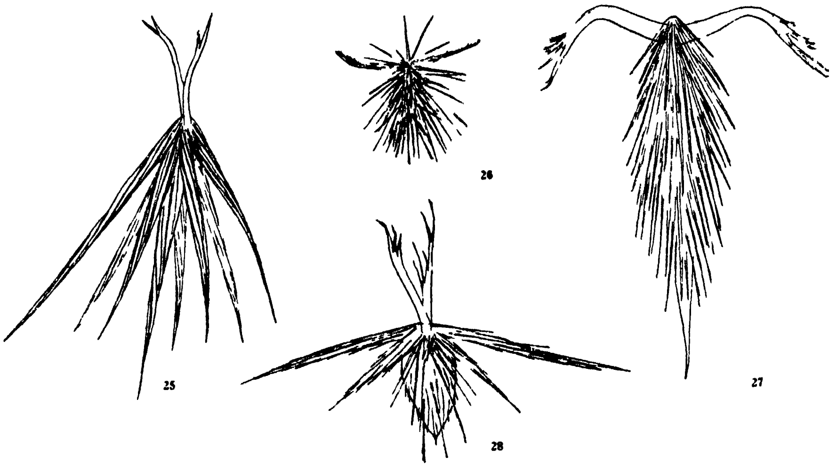
Plasmotomy is equal and longitudinal, the body while consideraly shortened retains its elongated form. The pellicular ridges are divided equally between the daughter cells and each takes the siderophile bar and flagellar bundle of its side (fig. 22). Neither the final stages of plasmotomy nor the reconstruction processes have been seen in fixed material. Living individuals with but one bundle and the opposite side flattened have been seen on numerous occasions (fig. 6), as have others with one large bundle paired with a very small one, the latter evidently growing out after recent division.

BEHAVIOR

In appearance and behavior *Hoplonympha natator* is perhaps the most striking of the flagellates of the termites. In the mass of organisms crowding the hind intestine of *K. simplicicornis,* its slender shape and peculiar methods of locomotion make possible a rapid movement from place to place even when crowding is so great as to bring all but the very small forms practically to a standstill. Its movements are very much those of the swimmer when using the breast stroke, but moving the arms alternately, never together. One flagellar bundle is drawn forward close to the body—with such great rapidity as almost to defy observation—by a contraction wave which moves along it from the base until the bundle is extended anteriorly (fig. 8, *a′-f′*). The bundle of the opposite side meanwhile, having completed its stroke, comes to lie posteriorly along the side of the body (fig. 8). The anteriorly directed bundle is now thrust laterally and backward with the motion of the swimmer's arm (fig. 8, *a-f*) and the opposite bundle drawn forward again. These movements are accomplished rapidly, the forward stroke even more rapidly than the effective back stroke. In a fluid medium this swimming movement is effective but in the crowded intestine where the bundle on the back stroke presses against the débris or the bodies of other protozoans, the organism is thrust violently forward and a very rapid, if jerky and irregular, progression obtained. The normal rate of the stroke seems to be about 60 times per minute. Individuals in artificial media have been observed in which the strokes occurred at the rate of 120 per minute. Numerous observations seem to show that under favorable conditions the body is moved forward on the average about one-half its own length at each stroke. This would mean a forward progression of thirty times its own length or approximately three millimeters per minute, about one-half the length of the hind intestine in which it lives. Actually it makes far less forward distance than this since it is constantly changing its course and meeting obstacles which slow it up or turn it back. It becomes apparent, however, that we are dealing here with an extremely powerful locomotor system, particularly effective under the normal conditions of the organism's environment.

The individuals of *H. natator* are ordinarily seen moving restlessly in a more or less direct course apparently entirely independent of one another or of local conditions within the intestine. On several occasions in artificial media, however, both within the unopened intestine or when free in the medium, they have been seen to congregate in great numbers in some limited area within or near the intestine and there execute a most striking dance of the nymphs, darting feverishly here and there in all directions but remaining within the general area. This would seem to be a typical motor reaction due perhaps to some chemical or pressure gradient.

In artificial media *H. natator*, after a very short time, ordinarily takes on a most peculiar and characteristic appearance simulated by no other protozoan on record unless it be *Polymastix,* which seems to show a similar tendency. The abnormal osmotic condition of the medium causes a shortening and thickening of the body. As the body is covered by a heavy, rigid, ridged, pellicle this means great mechanical stress with the peculiarly interesting result of a telescoping effect on the ridges and a consequent splitting off of long sliver-like portions (figs. 9, 27) along planes running from the surface of the ridge anteriorly and progressively deeper. These spine-like pellicular slivers all project posteriorly at a slight angle and give the animal the appearance of an ear of wheat or barley, or at times they recall the fiber rain-coats so common in the orient (fig. 27).



Figs. 25–28. Sketches of *Hoplonympha natator* gen. nov., sp. nov., from *Kalotermes simplicicornis* Banks, showing pellicular fimbriation and lobulation of body due to artificial media.

25 and 28. Variations in lobulation of body which at times replaces fimbriation (25), or accompanies it (28). × 850.

26. Showing complete disintegration which takes place at death in artificial media. × 530.

27. A characteristic stage in fimbriation of pellicle in which resemblance to an ear of grain is marked. × 1060.

So far as can be ascertained this condition never normally obtains within the termite intestine. Even in mitosis when the body normally shortens and thickens the process is accomplished without fracturing the pellicular ridges (fig. 22) probably because of the greater period of time involved. We are evidently seeing here, therefore, a mechanical reaction of the stiff pellicular coat to the rapid shortening of the body in the abnormal conditions of the artificial medium. It is interesting to consider that here was a possibility, perhaps inherent in these organisms since long before man came on the scene, whose expression was in all likelihood first made possible when the author examined the first fresh mount from this termite species in the Arizona cañon where they were taken. One is led to speculate on the appearance, full blown, of new characteristics inherent in the old conditions but awaiting new environmental conditions for their expression.

The organisms retain their locomotor activity for a long time after the fimbriation of the pellicular ridges. In a perfectly sealed vaseline mount many were still active after 72 hours. Aside from pellicular fimbriation their reactions to existence in artificial media are varied: Many individuals show a breaking up of the body into lobes (figs. 25, 28) ; others become greatly expanded in the vertical plane and often exhibit a spiral torsion of the body (fig. 7). At death the pellicular disintegration becomes complete (fig. 26). The rod-like form and more or less uniform size of the pellicular remnants (fig. 26) suggest the possibility that the pellicular armor may be laid down in the form of rods which later become associated to form a continuous sheath but retain their structural individuality.

The pellicular projections seem to be extremely rigid at first but after several hours in the medium, when the body is greatly shortened and the number of projections very great, they become flexible or at least moveable, probably due to the fimbriation of so much of the pellicular coat as to destroy its continuity and rigidity and allow the projecting fragments to move on the softer cytoplasm. Ordinarily a most rigid organism, *Hoplonympha* has been observed to become extremely flexible on rare occasions, under the comparatively normal conditions of a mount of the whole intestine without any distal fimbriation. This seems to be accomplished.by the separation of the pellicular armor into constituent rods which, while still individually rigid, move freely in or on the ectoplasm.

DISCUSSION

Hoplonympha stands in a position so isolated as to make it extremely difficult if not impossible to homologize its structures with those of any of the known hypermastigotes. In its development of a heavy and elaborately ridged pellicular armor and the accompanying rigid body form it has progressed along lines quite opposite to those followed by most if not all of the flagellates of the intestinal lumina of animals. The tendency among these latter Protozoa has been toward the development of a metabolic flexibility of body enabling them to move readily on or over one another and the débris of their habitat. They have tended toward the development, in the holozoic forms at least, of a large cytosome and a very thin periplast allowing for the ingestion of relatively large food masses. *Hoplonympha natator* or one of its ancestral forms, on the contrary, having given up the holozoic habit, was relieved of the necessity for a relatively large cytosome and has been enabled to develop the long slender form which means both a great increase in absorptive surface and at the same time a great decrease in friction surface due to the small vertical cross-section and the stream-line body shape particularly suitable for rapid progression through a liquid medium or through the interstices of the intestinal contents. Freed from the necessity of maintaining a thin periplast to allow for the ingestion of food, *Hoplonympha* has developed a remarkable pellicular armor which, providing a rigidity of body favorable to forceful movements and at the same time a protection against the dangers incident upon rapid movement through a débris filled intestine, has made for a very perfect adaptation of the organism to the environment. The elongation of body which we find in *Streblomastix strix* Kofoid and Swezy, also a non-xylophagous form, is evidently an adaptation to the need of absorptive surface, but *Streblomastix* has given up the actively motile habit for that of attachment to the body wall and there has been therefore no pellicular development. Neither the neuromotor systems of the two forms nor their mode of division suggest any relationship and one is forced to the opinion that the elongation of the body which is their only common character is a common response to the need of absorptive surface, i.e., that we have here convergence or parallel evolution rather than indications of immediate phylogenetic relationship.

Among the hypermastigotes *Hoplonympha* shows relationship to the higher families rather than to the Lophomonadidae by the equal division of the neuromotor structure between the daughter organisms. Structurally its relationships among the higher forms are with the Staurojoeninidae on the one hand and the Trichonymphidae on the other. Like *Staurojoenina* the flagella are in opposed bundles arising from flagellar plates located at the anterior end of the body. In

Hoplonympha these are paired and accompanied by other bilaterally arranged structures while in *Staurojoenina* these structures are in fours. Other possibly homologous structures are the paired rhizoplast bands and the threads running forward from the nuclear poles in *Hoplonympha* at the time of division which seem homologous with the rhizoplast bands of *Staurojoenina* (Kirby, 1926). The paradesmose of *Hoplonympha* is also comparable in shape and position to that of *Staurojoenina.*

The anterior cap of *Hoplonympha* and the granular column suspending the nucleus are suggestive of similar structures in the highest hypermastigotes (*Trichonympha, Pseudotrichonympha, Teratonympha*) but in the light of our present knowledge this must be considered as an adaptation to the same environmental needs. If the pellicular system can be shown to be a high development of a system of rodlets we have another homology with *Staurojoenina* in whose periplast Kirby (1926) finds numerous tiny rodlets. This seems to be a widespread character among the Protozoa of termites, however, occurring in *Devescovina* as well as in certain hypermastigotes.

The bilaterality of *Hoplonympha* so strongly brought out by the position of the flagellar bundles, their basal plates, and the lateral vesicles is one of its most striking morphological characteristics. Kofoid and Swezy (1926) have recently described the bilateral arrangement of the flagellar mechanism in *Oxymonas* where there are two sets of *Monocercomonas*-like neuromotor systems integrated by an interblepharoplast rhizoplast. This gives us some suggestion as to how bilaterality may have arisen but it would seem premature to predicate any necessary phylogenetic relationship between the polymastigote *Oxymonas* and the highly developed hypermastigote *Hoplonympha.*

Polymastix melolonthae (Alexieff) and to a less degree *P. bufonis* (Dobell) present characters strikingly suggestive of a possible relation to *Hoplonympha* in the bilateral arrangement of their flagella and blepharoplasts (Mackinnon, 1913; Swezy, 1916), which may very well be merely a premitotic phenomenon (Swezy, 1916), and in the developed pellicle with pellicular ridges, "ribs," "striae" (Mackinnon, 1913, and Swezy, 1916). Mackinnon's description and figures (1913, p. 301, pl. 18, figs. 10–16) make clear that she found in *P. melolonthae* not only a similar pellicular structure but also a tendency to pellicular fimbriation and to a lobulation of the body in artificial media strikingly suggestive of that here described for *Hoplonympha.*

Mackinnon sought to show by finding homologies between *Polymastix* and *Lophomonas* that the Hypermastigina are of polymastigote origin with strongest affinities with the Polymastigidae. The relationship between *Polymastix* and *Lophomonas* seems slight, but there seems more than a suggestion of common inheritance between *Polymastix* and *Hoplonympha* belonging to the two different orders as they do. Indeed from what little data we have at hand one may well wonder if the order Hypermastigina is not a heterogeneous group, all derived no doubt from polymastigote ancestors but by several different lines emerging by parallel evolution under the influence of approximately the same set of environmental conditions, those found in the intestine of insects.

SYSTEMATIC POSITION

Hoplonympha natator, therefore, while probably distantly related to *Polymastix* among the polymastigotes and less distantly to *Staurojoenina* among the hypermastigotes, presents unique structural characters which necessitate the erection of a new family to receive it.

Family HOPLONYMPHIDAE, fam. nov.

Diagnosis.—Hypermastigotes characterized by bilateral symmetry emphasized by locomotor organs in the form of two laterally placed flagellar bundles each arising from a flagellar plate near the anterior end of the slender body which is protected by a highly developed pellicular armor, and by an equal division of neuromotor structures in binary fission.

Type genus.—**Hoplonympha** gen. nov.

Hoplonympha gen. nov.

Diagnosis.—Slender bodied Hoplonymphidae with thick, rigid, pellicular armor, thrown into linear ridges with a slight leiotropic twist. Each flagellar bundle is made up of numerous flagella integrated and acting as a unit under normal conditions. At the anterior tip of the body is a tiny vesicle, the cap, containing chromatic elements resting on an irregular siderophile body tentatively designated here, the blepharoplast. From the blepharoplast arise the flagellar plates as well as the rhizoplast bands which run to the nucleus. The nucleus is near the anterior end of the body suspended by a column of specialized granular cytoplasm.

The neuromotor structures are distributed equally in mitosis.

Type species.—*H. natator* sp. nov., from the hind intestine of *Kalotermes simplicicornis* Banks, from Texas, Arizona, southern California, and Lower California.

SUMMARY

Hoplonympha natator gen. nov., sp. nov., a non-xylophagous hypermastigote inhabiting the hind intestine of *Kalotermes simplicicornis* Banks shows marked adaptations to its environment and to its metabolic habit. The elongated, stream-line body provides a maximum of surface for absorption with a minimum of resistance to progression. A highly developed and effective locomotor system, consisting of two, alternately stroking, opposed, flagellar bundles, allows for rapid progression through the crowded intestine. A heavy pellicular armor, thrown up into permanent, linear ridges with a slight leiotropic trend, gives the body a rigidity favorable to effective locomotion and provides protection against the dangers incident upon rapid progression through a débris-filled intestine.

Its distinctly bilateral organization, together with the possession of a heavy, rigid, pellicular armor, are peculiar characteristics which mark the new genus *Hoplonympha* as the type of a new family in the Order Hypermastigina agreeing in its type of division with the higher hypermastigotes as contrasted to Lophomonadidae and structurally most closely related to Staurojoeninidae Grassi

The shortening of the body of *Hoplonympha natator* under the conditions of artificial media results in an amazing fimbriation of the pellicular armor leading to its ultimate disintegration into rod-like elements. This pellicular armor and the phenomena of its disintegration described above seem to indicate affinities between this hypermastigote and the polymastigote, *Polymastix melolonthae* (Alexieff). This apparent relationship would seem to add to the accumulating evidences for the polyphyletic nature of the Order Hypermastigina as at present constituted.

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ON METADEVESCOVINA DEBILIS GEN. NOV., SP. NOV.

A XYLOPHAGOUS POLYMASTIGOTE FROM THE TERMITE, KALOTERMES HUBBARDI BANKS

BY

S. F. LIGHT

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A XYLOPHAGOUS POLYMASTIGOTE FROM THE TERMITE, KALOTERMES HUBBARDI BANKS

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S. F. LIGHT

Metadevescovina debilis gen. nov., sp. nov., is the dominant species in the intestinal fauna of the termite, *Kalotermes hubbardi* Banks (1920) of Arizona and California. Its only large associate there is *Staurojoenina* sp.

Kalotermes hubbardi presents in most extreme form that hypertrophy of the third antennal segment of the soldier caste which characterizes most, perhaps properly all, of the species of the genus *Kalotermes* s.s. In the exaggeration of this character as well as in its protozoan fauna it is sharply differentiated from all other known species save *K. minor* Hagen of approximately the same range which shows this character in only less degree than *K. hubbardi.* The two large Protozoa found in *K. minor* are *Staurojoenina assimilis* Kirby (1926) and a species of *Devescovina* (*fide* Kirby), the genus most closely related to *Metadevescovina.* This striking correlation between morphological similarity and similarity of protozoan faunas would seem to point to a distinct phylogenetic significance for these faunas and, by inference, for those of other termite species.

My attention was first called to the form described in this paper by Dr. Harold Kirby, Jr., now of Yale University, to whom I had sent specimens of *K. hubbardi* from the field to enable him to compare the *Staurojoenina* in it with that of *K. minor* which he was studying at the time. He at once noted that the form associated with *Staurojoenina* was similar, in superficial appearance at least, to *Caduceia theobromae* Franca (1918).

I wish here to express my thanks to Dr. C. A. Kofoid, with whom this work was done, for his constant aid and advice. Material with which to carry on the work was received through the kindness of

Mr. Henri Behoteguy of Phoenix, Arizona, and I wish to take this opportunity to thank him for this and for aid while in the field. Thanks are also due to Dr. Olive Swezy for aid and suggestions in the preparation of the illustrations.

Material was studied both fresh and in the form of stained smears. The usual fixing and staining methods were followed. Fresh material was studied in dilute Locke's solution. When the living organisms were treated with a very weak aqueous solution of picric acid, a weak tincture of iodin, or a weak alcoholic solution of eosin, certain structures were seen to greater advantage, particularly the flagella. The bacterial nature of the attached bacteria, spirilla, or spirochaetes, was thus made clear—otherwise these were easily mistaken for lateral flagella. The picric acid when in proper dilution renders the cytoplasm wonderfully transparent and makes visible the relations of certain parts of the neuromotor system otherwise seen but dimly in life.

Metadevescovina debilis sp. nov.

Plate 10, figures 1–12; figures A, B, C

Form and size.—*Metadevescovina* is 45 (30–70) microns in length and 20 (15–30) microns in greatest diameter. The body varies considerably in shape, size, and relative proportions. In fixed specimens it is usually elongated oval (pl. 10, fig. 3; fig. A). In life it is typically somewhat spindle-shaped, ovoidal in contour, circular in cross-section, more bluntly rounded in front, tapering from near the anterior one-third to a rather slender, pointed, posterior tip (figs. B, C) from which the end of the axostyle may project in individuals, in artificial media (figs. B-4, 5). This contour is not symmetrical since as a rule the thickest part of the body is considerably in front of the middle and since the anterior end is marked by a notch on the side from which the flagella take their origin (fig. B-2). This side will be designated as ventral since it seems to correspond in position to that of the cytostome in other flagellates. Right and left, dorsal and ventral, as used hereafter for this species are based on this assumed position.

Nucleus.—The nucleus is unique in shape and position. It is permanently located very near the anterior end of the body, elongated dorsoventrally and pulled out ventrally into a long, slender, gracefully curved neck (pl. 10, figs. 1,6). The nucleus lies on the enlarged anterior end of the axostyle, surrounded by the elevated margins of the cup-like, transverse lamella (*tr. lam.*, fig. A). A projection from the mid-posterior surface of the nucleus clearly rests in a concavity in the center of the anterior end of the main region of the axostyle (pl. 10, figs. 1, 6, 11, 12). The neck of the nucleus runs ventrally and somewhat anteriorly over the surface of the axostylar extension to connect with one of the blepharoplasts, in most cases apparently the primary (fig. A; pl. 10, fig. 1), in one case at least the secondary (pl. 10, fig. 12). The chromatin of the nucleus ordinarily appears diffuse and stains lightly and the limits of the chromatic region are often vague. No definite peripheral clear zone has been made out. Two siderophile, spherical granules, probably karyosomes, are usually to be made out surrounded by clear halos (*kary.*, fig. A; pl. 10, fig. 1). While the interphase nucleus of *Metadevescovina* seems to show no relationship to that of the other flagellates of termites, nevertheless in mitosis it shows the clear area about the chromatin, retains the nuclear membrane throughout division with centrosomes located on the nuclear membrane, and shows the paradesmose in typical relations (pl. 10, fig. 8).

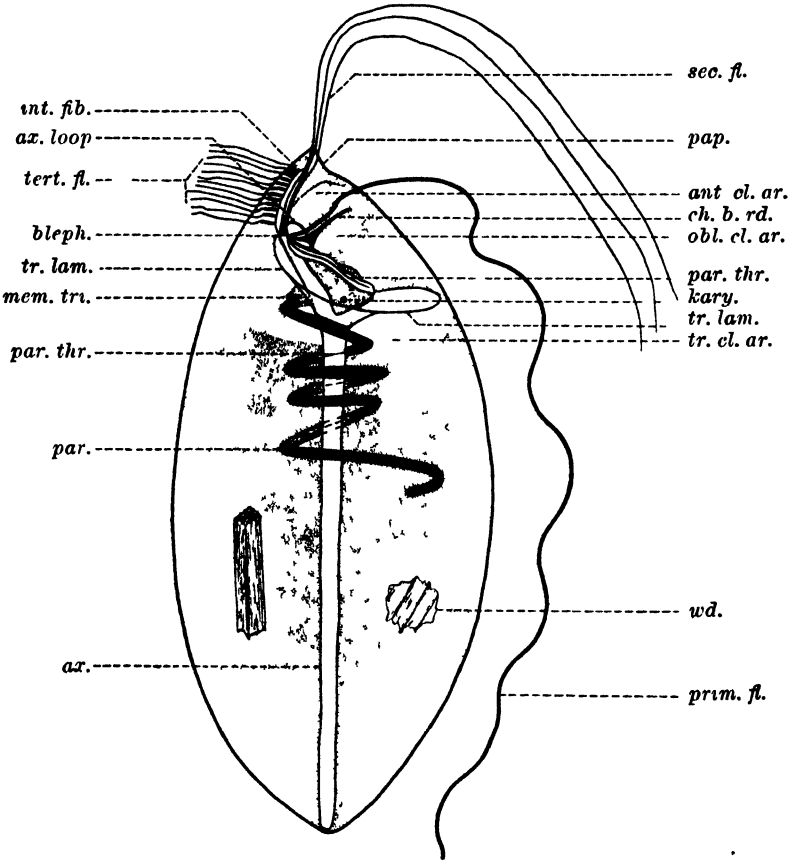

Fig. A. Diagrammatic figure of *Metadevescovina debilis* gen. nov., sp. nov., from *Kalotermes hubbardi* Banks, in ventro-sinistral view. × 2600.

Abbreviations: *ant. cl. ar.*, anterior clear area, moved from normal position to the right of secondaries, the anterior internal membrane bounding its left side; *ax.*, axostyle; *ax. loop*, loop of the axostyle; *bleph.*, blepharoplasts; *ch. b. rd.*, chromatic basal rod; *int. fib.*, intracytoplasmic fibril; *kary.*, karyosome in clear halo; *mem. tri.*, membrane of dense triangle at ventral angle of triangle where it forms a trough for the parabasal; *obl. cl. ar.*, oblique clear area; *pap.*, papilla; *par.*, parabasal apparatus; *par. thr.*, parabasal thread; *prim. fl.*, primary flagellum or trailer; *sec. fl.*, secondary flagella; *tert. fl.*, tertiary flagella; *tr. cl. ar.*, transverse clear area; *tr. lam.*, transverse lamella; *wd.*, wood inclusion.

Neuromotor System.—The neuromotor system of *Metadevescovina* is comparatively complex and certain points as to relations of parts are still obscure. It consists of four large, free, flagella which may be designated as three secondary flagella (*sec. fl.*, fig. A) and a primary flagellum (*prim. fl.*), each with a granule at its base, the blepharoplasts (*bleph.*), a number of tertiary flagella (*tert. fl.*) arising from an intracytoplasmic fibril (*int. fib.*), a chromatic basal rod (*ch. b. rd.*) underlying the base of the primary, a large, rigid axostyle (*ax.*) extending throughout the body, and a long parabasal (*par.*) coiled about the nucleus and the axostyle in the form of a left handed spiral. Although still somewhat obscure it seems probable that the anterior internal membrane and the membrane of the triangle (*mem. tri.*) are parts of the neuromotor system.

Blepharoplasts.—With considerable hesitation the designation blepharoplasts has been given to the relatively minute and inconspicuous granules at the bases of the four large flagella for which no surrounding vesicle, such as found in *Devescovina,* has been made out. There remains the possibility that the structure termed here the neck of the nucleus may be a vesicular blepharoplast of a peculiar type in such close association with the nucleus as to render its delimitation very difficult.

Integrations.—The structures are so crowded in the region of the blepharoplasts and their staining reactions are such as to make it extremely difficult to make out the actual immediate integrations of the axostyle, the flagella, the internal membranes, and the parabasal with the blepharoplasts. That actual connections exist can be occasionally seen in specimens with especially favorable stain and lying in a favorable position (pl. 10, figs. 11, 12).

The parabasal is connected to the primary blepharoplast by means of the parabasal thread (pl. 10, fig. 12). The axostyle ends in a loop whose marginal fiber seems to join the fiber which forms a connecting link between the blepharoplasts (fig. A; pl. 10, fig. 11) joining them in series with the inner end of the chromatic basal rod. The exact connections of the intracytoplasmic fibril have not been made out but it seems to arise somewhere near the quaternary blepharoplast. The origin of the centrosomes which are found on the nuclear membrane at either end of the paradesmose during mitosis (pl. 10, fig. 8) has not been made out.

Flagella.—The flagella are of three types which we may designate the primary flagellum (*prim. fl.,* fig. A) or trailer, the secondary flagella or anterior flagella (*sec. fl.*), and the tertiary flagella (*tert. fl.*). The latter arise from an intracytoplasmic fibril (*int. fib.*) which lies parallel to the intracytoplasmic portion of the anteriormost secondary.

The primary flagellum, which is considerably larger than the secondaries, takes its origin very near them just below and slightly to the right of the right end of the chromatic basal rod (fig. A), at the right hand margin of the ventral notch. Thence it runs, when at rest, to the left and somewhat posteriorly along the posterior margin of the notch, parallel and ventral to the chromatic basal rod, leaving the cytoplasm near the left side of the notch. Thence it is usually directed to the left and posteriorly. It is considerably longer than the body and of the same thickness throughout, considerably more slender than the corresponding flagellum in *Devescovina.*

The three secondaries arise close together just anterior and to the right of the right end of the chromatic basal rod (fig. A). They lie close together, basally, shallowly imbedded in the cytoplasm near the ventral surface, running when at rest to the left and somewhat anteriorly along the anterior margin of the notch to emerge near the left side of the notch. Thence they are directed more or less anteriorly and to the left. When the return beat is completed they are directed almost directly anteriorly, their bases carrying with them a small pointed cytoplasmic projection (*pap.*, fig. A), designated the papilla by Grassi in *Macrotrichomonas.* When the functional down stroke is completed their bases lie almost straight across the ventral surface (fig. B, 3).

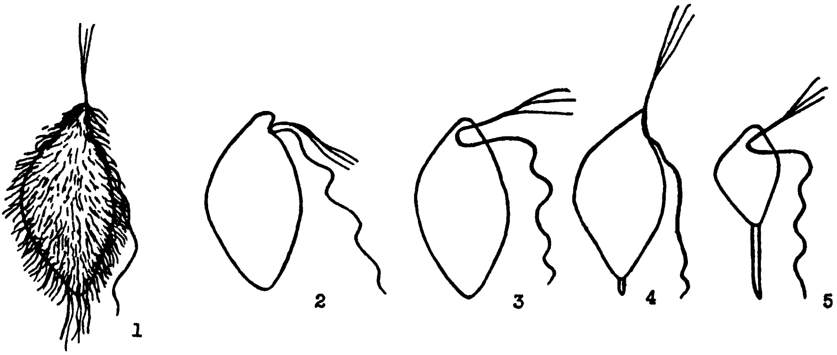

Fig. B. Outline diagrams of *Metadevescovina debilis* gen. nov., sp. nov., from life. × 500.

1. Organism on its left side. Secondaries at completion of return beat, primary at completion of effective stroke, papilla distinct, notch practically obliterated. Note covering of adherent bacteria.

2. Same position but with flagella approximated as at completion of effective stroke.

3. Ventral side up. Showing the appearance of continuity of bases of secondaries and primary common in fixed and living material.

4, 5. Showing the axostyle projecting in varying degree as seen in artificial media.

The bases of the major flagella originate very close together and near the end of the chromatic basal rod. Hence in many views the rod forms a continuous background which obscures the endings and we seem to have a single flagellum attached by its U-shaped middle portion (fig. B, 3). Such is the impression often gained from the living as well as fixed material, particularly at low magnifications. A similar condition in *Caduceia* might explain Franca's figures of the number and relations of the major flagella in that form (1918).

The tertiary flagella (*tert. fl.*, fig. A) are short, rather thick for their length, and apparently comparatively stiff. They are about twelve in number, originating from a fibril in the cytoplasm which parallels the course of the intracytoplasmic portion of the secondaries and lies so close to them as to give the impression in many specimens that the tertiaries arise from the outermost secondary (pl. 10, fig. 12). It has been impossible to trace this fibril to definite endings. Distally it usually seems to run under the bases of the secondaries near the point of their emergence from the cytoplasm (fig. A). Posteriorly it runs to near the neuromotor center beyond the quaternary blepharoplast and seems in numerous instances to have a close relation with the loop of the axostyle. This may be contiguity, however, rather than continuity.

The tertiaries have thick bases within the cytoplasm (fig. A). Their free portions seem to bend dorsally at the point of emergence, i.e., toward the body. They simulate closely the adherent spirilla but their constant presence, their definite intracytoplasmic bases and regular arrangement, and their attachment to the intracytoplasmic fibril make it certain that we are dealing here with a definite part of the neuromotor system although its function is as yet entirely problematic.

Axostyle.—The axostyle (*ax.*, fig. A) is relatively large, extending from the neuromotor center at the anterior end of the body to the posterior tip of the body. In living specimens within the termite intestine it has not been seen to protrude but in specimens kept for any length of time in artificial media it projects more or less prominently from the posterior end of the body (fig. B, 4). In degenerating individuals where extensive cytolysis has taken place the axostyle may be seen to project for two-thirds to four-fifths of its length as a conspicuous, transparent, rigid, homogeneous-looking, slightly bent or straight rod (fig. B, 5). It does not stain as a rule and is often invisible or seen with difficulty in stained material. Its central portion shows a faintly fibrillar structure (pl. 10, fig. 6) which ends at the nucleus. It has a thin but distinct surrounding membrane which is more conspicuous anteriorly and ventrally where it runs into the loop. This membrane becomes very distinct at the time of mitosis, when it persists after the new axostyles have formed (pl. 10, fig. 9). Posterior to the nucleus the axostyle is a straight or gradually tapering rod, more or less abruptly pointed or rounded at the tip. Its center supports the nucleus, a posterior projection of which fits into a concavity in the center of the axostyle (pl. 10, fig. 6). At this level it is closely associated with the transverse lamella which gives the appearance of a broad, shallowly funnel-shaped, expansion of the axostylar substance. This would seem to be merely an apparent connection, however, since in certain specimens in which the axostyle takes a faint stain it seems separate from this structure (fig. A; pl. 10, fig. 1). The axostyle continues past the nucleus as a somewhat broader flattened structure running ventrally under the neck of the nucleus and then curving around it to the right in the form of a loop which arches and turns posteriorly and ventrally to connect with the neuromotor center (fig. A, *ax. loop*, pl. 10, figs. 6, 11).

Parabasal apparatus.—The parabasal apparatus (*par.*, fig. A) is large, longer than the body, apparently firm in consistency, with a denser, more deeply staining central thread, the parabasal thread (*par. thr.*, fig. A) of Janicki (1915), which integrates with the blepharoplast (pl. 10, fig. 12). The parabasal coils in rather wide, often angular coils, about the nucleus and axostyle in a leiotropic spiral, i.e., passing anteriorly from right to left over—and from left to right under the nucleus and axostyle. It arises from the primary blepharoplast, i.e., near the ventral surface at the anterior end of the body, runs posteriorly and dorsally along the left side of the neck of the nucleus (pl. 10, fig. 12), curves up and to the right over the posterior region of the nucleus just anterior to the transverse lamella (fig. A; pl. 10, fig. 12) and down on the right side through the triangle of dense cytoplasm the membrane of which forms a trough for it in the region of its sharp ventral angle (pl. 10, figs. 3, 4, 6). Emerging from the trough of this membrane it passes diagonally to the left and posteriorly under the axostyle into the transverse clear area and thence up and posteriorly, then to the right over the axostyle and ventrally and posteriorly into the granular cytoplasm of the posterior region of the body, where it makes three to five loops about the axostyle to end freely in the cytoplasm far from the axostyle (fig. A; pl. 10, fig. 2, 3, 12), usually behind the middle of the body. In living individuals as many as seven loops are occasionally seen (fig. C, 3). The portion of the parabasal anterior to the point where it emerges from the triangle of dense cytoplasm is extremely obscure in most individuals. Only occasionally does this anterior portion show at all in specimens stained in iron haematoxylin (pl. 10, fig. 12). The entire parabasal stains but poorly with this stain and is often nearly or quite invisible. In Delafield's haematoxylin the parabasal stains deeply and other structures but little and one sees clearly the anterior continuation of the parabasal.

Cytoplasm.—The cytoplasm in fixed condition contains granules in a more or less conspicuously alveolar meshwork throughout save for a series of areas of transparent cytoplasm which are designated clear areas and described below. There is no visible differentiation into ectoplasm and endoplasm, although the cytoplasm near the margin appears more granular in fixed specimens, nor is there a structurally visible periplast although the organism retains an unusually constant form in life.

Scattered through the cytoplasm behind the transverse clear area to be described below are numerous small wood fragments (pl. 10, fig. 3) and usually a few of the larger size (*wd.*, fig. A), but never any large enough to distort the body such as one sees in the large hypermastigotes. Spherical, clear vacuoles seen in the cytoplasm of individuals kept in artificial media seem to represent degeneration phenomena. Spheres containing numerous small, deeply staining bodies are frequently seen in the posterior cytoplasm and apparently are parasites (pl. 10, fig. 3). The parasitized individuals seem often to reach a larger size than that normal for the species.

Internal membranes.—The name internal membranes is used to designate an apparently continuous structure whose parts have been designated respectively the membrane of the dense triangle (*mem. tri.*, fig. A) and the anterior membrane. In connection or continuity with the former posteriorly is the transverse lamella (*tr. lam.*) which is actually or apparently continuous with the axostyle in the region of its expansion to support the nucleus and appears in many specimens to be in the form of an axostylar cup (pl. 10, figs. 1, 2, 12). In favorable living material this may be seen to be a very thin, rigid structure, sharp in optical section, moving with the axostyle (fig. C, 3). The relations of these so-called membranes are extremely difficult to make out since they do not take the stain and are with difficulty differentiated from the cytoplasm. They lie, furthermore, in a region already complicated by the presence of other structures and take a more or less spiral course about the nucleus and hence are to be made out only by means of a series of optical sections. Most conspicuous is the membrane of what we may call the dense triangle (*mem. tri.*, fig. A; pl. 10, figs. 3, 4, 7). Located on the right side of the anterior end of the animal near the surface, this triangle seems to consist of rather dense homogeneous cytoplasm external to the membrane which forms a trough, concave to the surface (pl. 10, figs. 3, 4). The anterodorsal and the posterior margins of this triangle are very sharp where the membrane approaches the surface (pl. 10, figs. 3, 4, 7). They border the oblique and transverse clear areas, respectively, which will be described later. These margins diverge dorsally considerably beyond the limits of the dense cytoplasm. The central portion of the membrane seems to pass around the dorsal end of the nucleus with the parabasal (pl. 10, figs. 3, 4), leaving a more or less clear zone beyond, i.e., dorsal to the nucleus (pl. 10, fig. 6). The anterior margin may be seen, by careful focussing in particularly favorable specimens, to be continuous with the left margin of the anterior clear area (pl. 10, fig. 3) to be described below that is with the anterior membrane which seems to form the left wall of that clear area. The ventral angle of the triangle is blunt and the membrane dense and deeply grooved where it forms a trough, opening externally, which curves down around the ventral margin of the axostyle (pl. 10, figs. 3, 6). Through this trough the parabasal runs up into the dense triangle (pl. 10, figs. 3, 4) and continues along the posterior portion of its ventral two-thirds before it turns to the left to run over the dorsal surface of the posterior region of the nucleus (pl. 10, figs. 3, 12). The posterior border of the membrane of the triangle appears to be attached to—or continuous with—the dorsal portion of the right margin of the transverse lamella (fig. A; pl. 10, figs. 1, 2).

Clear areas.—Closely associated with the internal membranes and the transverse lamella is a series of peculiar, perfectly transparent, apparently structureless, clear areas which give the anterior end of the organism a characteristic appearance (pl. 10, figs. 1–7). These are so clear as to give the impression of empty cavities. When such an area approaches to the very periplast, as does the anterior clear area, it has a most misleading resemblance to a cytostome (pl. 10, fig. 5). These clear areas are entirely invisible in the living organism, however, and nothing in the nature of a cytostome is to be made out.

These areas are continuous (pl. 10, fig. 7) and have in general a spiral arrangement. The anterior clear area (*ant. cl. ar.*, fig. A; pl. 10, fig. 5) is narrow and slit-like at its anterior end but broadens internally almost at once. Superficially it is elongated in a generally dorsoventral direction, running downward from right to left, its lower end at the surface lying near the anterior margin of the intracytoplasmic bases of the secondary flagella, and hence it is overhung by the tertiary flagella. Its left margin is convex and very sharp, being the margin of a part of the internal membrane. Its right margin is characteristically biconvex. On focussing down this clear area is seen to contract internally and to connect with two other clear areas. Its more ventral portion is continuous with a less well defined clear area which lies along the anterior surface of the neck and body of the nucleus (pl. 10, fig. 6). The other connection is over the dorsal margin with the oblique clear area (*ob. cl. ar.*, fig. A; pl. 10, fig. 3), which runs posteriorly and ventrally along the anterior margin of the dense triangle and connects around the ventral angle of the triangle (pl. 10, fig. 7) with the large transverse clear area (*tr. cl. ar.*, fig. A; pl. 10, figs. 1–4, 6, 7). This lies behind the transverse lamella, occupying a transverse disk-shaped region, oval in optical section, cutting off the anterior from the posterior area of granulated cytoplasm save for a ventral bridge of granulated cytoplasm (fig. A).

It is significant that each of these clear areas is bounded on one side by a membrane; the anterior clear area on its left side by the anterior internal membrane, the oblique clear area by the anterior margin of the membrane of the triangle and the transverse clear area by the posterior margin of the membrane of the triangle and broadly by the transverse lamella.

Macrotrichomonas Grassi (1917) has a remarkably active internal membrane which executes powerful movements, churning up the cytoplasm in an amazing manner. It is significant that along the borders of this membrane in *Macrotrichomonas* and underlying it we find in fixed material areas of clear protoplasm strikingly similar to those in *Metadevescovina*.

These clear areas and the membrane cannot be seen in life in *Metadevescovina* where the cytoplasm is opaque, but neither can they be seen in *Macrotrichomonas* in life, which is the most transparent of organisms, although the churning of the cytoplasm and the rotation of the whole neuromotor system due to the powerful movement of the internal membrane are quite apparent.

These considerations make it probable that the clear areas are regions of protoplasm kept more or less continually churned up by active movements of internal structures, possibly those which we have designated the internal membranes and by the passive movements of the transverse lamella. Certain observations on unusually transparent living specimens massed together, given later, favor this conclusion. The transverse lamella is clearly rigid, but it is kept moving up and down by the more or less violent movements of the papilla and secondaries and by the movements of the anterior end of the axostyle. Neither the membrane of the triangle nor the anterior membrane can be made out in life, but it seems probable that one or both of them have to do with the movements of the tip of the body and with violent whirling of the whole neuromotor system to be discussed under behavior.

MITOSIS

The material available does not allow for a complete description of the phenomena of mitosis in *Metadevescovina.* Suffice it to say at this time that the process is typical of that for the group. The nucleus takes on the typical vesicular form, a paradesmose is produced ending in centrosomes on the nuclear membrane, which is retained throughout the process (pl. 10, figs. 8–10). The chromosomes are few in number, probably four (pl. 10, fig. 10).

The history of the major flagella and the chromatic basal rod is not known, but they are already present in late prophase and are seen to arise from a blepharoplast connected to the centrosome by a rhizoplast (pl. 10, fig. 8). The evidence as to the origin of the parabasals and axostyle is not conclusive, but points to their origin by outgrowth, the former from the centrosome and the latter from the blepharoplasts, if the fibrils shown in figure 8 of plate 10, running obliquely to the right from each blepharoplast, are rightly interpreted as the new axostyles. How easily one may mistake this origin for one by splitting, is illustrated by specimens such as the one represented in figure 9 of plate 10, in which the remnants of the old axostyle give the appearance of a posterior unsplit portion of a dividing axostyle.

APPEARANCE AND BEHAVIOR

Under all ordinary conditions *Metadevescovina* is found covered with somewhat regularly spaced spirilla-like bacteria (fig. B, 1; pl. 10, figs. 2, 5, 7), which have been called spirochaetes by Dobell (1910) and Damon (1926). These are very easily mistaken, particularly in fixed material, for flagella of the type found on the bodies of numerous hypermastigotes. Careful observation, however, shows that they are thicker in proportion to their length than is true of most flagella. In life their movements are seen to be without any synchronization, loosely spiral undulations as if they were attempting to free themselves. These bacteria present a most bizarre picture wriggling and writhing in all directions like Medusa's hair. It has been found that treatment with a weak tincture of iodin in 70 per cent alcohol will free the body of most of them and thus facilitate study of the flagella.

Metadevescovina differs from many of the Protozoa of the termites in that it is opaque in life, under ordinary conditions showing nothing of its internal structure. The axostyle and the loosely coiled parabasal apparatus can be seen in life in occasional individuals under the abnormal conditions in an artificial medium or after treatment with dilute picric acid. The nucleus is not visible in unfixed individuals.

As the specific name indicates, *Metadevescovina debilis* is apparently capable of but little active locomotion. The locomotor apparatus consists essentially of the four major flagella. So far it has not been possible to make out any normal activity on the part of the tertiaries. Their position on the slender, labile, anterior tip (papilla), surrounded by constantly moving, attached bacteria makes it very difficult to get a view of them while the animal is normally active. When they have been made out, even though in individuals whose secondaries were still beating vigorously, they showed little movement but presented a comparatively stiff appearance, all curved in approximately the same manner (fig. A; pl. 10, fig. 12), or at most, they show spasmodic movements of the tips.

The primary flagellum simulates more or less closely in position and activity, the trailer of *Devescovina.* It is ordinarily directed to the left and posteriorly and has an ineffective beat which does not move the flagellum as a whole but passes along it, giving to it an undulatory motion very suggestive of the motion of the flagellum of the undulating membrane of certain polymastigotes. The base of the primary moves backward and forward within the cytoplasm in a very narrow arc.

The secondaries seem to be the only effective locomotor apparatus and they but slightly so. They ordinarily beat stiffly back against the ventral and left sides of the body at the rate of about 60–80 strokes per minute in specimens in Cleveland's solution. On the forward, i.e., the return stroke, the bases of the secondaries move forward, carrying with them a nose-like papilla of cytoplasm until just before the effective stroke they point directly anteriorly (fig. B, 1). At the completion of the effective stroke they are directed almost transversely to the left across the ventral surface, coming very close at this moment to the base of the primary (fig. B, 2; fig. C, 3). In fixed material they seem to have as a rule a position somewhat more distant from the primary (fig. B, 3). They are able to move the body in a jerky, ineffective manner, to cause the body to rotate slowly in either direction and even to accomplish very slow forward progression, particularly in their normal habitat where the intestinal débris seems to furnish fulcra against which the secondaries push in moving the body forward.

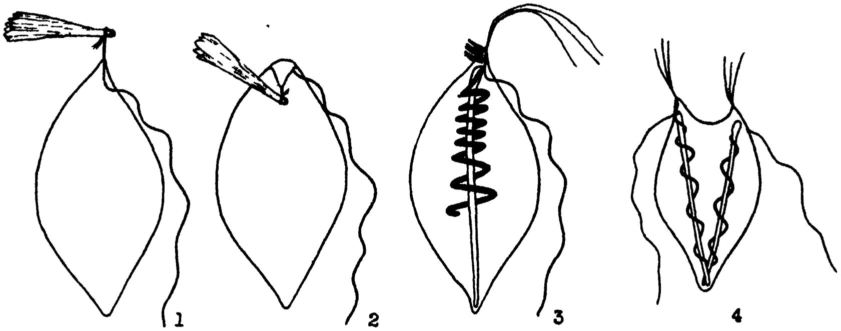

Fig. C. Sketches of *Metadevescovina debilis* gen. nov., sp. nov., from life. × 800.

1, 2. An individual with secondary flagella entangled about a piece of débris. 1, position on relaxation of secondaries, and 2, position at completion of effective stroke of secondaries.

3. After treatment with very dilute picric acid, showing maximum of structure visible in life. Note the transverse lamella, the numerous coils of the parabasal about the axostyle and the tertiary flagella.

4. An individual with completely divided nucleus and neuromotor system. Compare separately coiled parabasals with an earlier condition, figured in plate 10, fig. 9.

The papilla may be seen at times to be moved about in all directions with a nervous, jerky, movement suggestive of the sensitive movements of the nose of a nibbling rodent. The secondaries while typically beating as described may beat in any direction. Sometimes they are seen to make several convulsive half beats before returning to the anterior position. A specimen was seen whose secondaries had become entangled in the end of a piece of débris (fig. C, 1) and which gave the impression of struggling to free itself. At the completion of the stroke the papilla could be seen to be turned back over the ventral surface of the body, carrying the end of the piece of débris back against the surface of the body (fig. C, 2). In this position there would often be several spasmodic contractions before relaxation and the return beat. The fragment was so balanced that as soon as the pressure was released it fell back into the original position again. The whole body was moved by the contractions. This was not merely a beating of flagella but of their intracytoplasmic bases and of internal vibratile structures (the internal membranes?) which could be seen to make sudden convulsive movements by no means always synchronous with the movements of the flagella.

In a fresh mount from the intestine of a termite kept for many weeks on filter paper the organisms were wonderfully clear, the axostyle, parabasal body, and transverse lamella showing with the utmost distinctness (fig. C, 3). Where crowded together a phenomenon was noted which is very commonly seen also in *Macrotrichomonas* when in artificial media. The whole neuromotor system, with the exception of the posterior portion of the parabasal body, would suddenly rotate several times in one direction. This was usually followed by a pause and a subsequent rotation in the opposite direction. In other cases the rotation was continued in one direction with the result that the posterior portion of the parabasal was unwound and became a tangled mass in the cytoplasm. This does not occur in *Macrotrichomonas*, where the parabasal is wound very tightly about the axostyle.

The anterior portion of the parabasal remained absolutely fixed in position throughout these violent movements resembling exactly the anterior end of a wound spring, with the transverse membrane lying between the first two turns of the spiral (fig. C, 3). The cause of this movement in *Macrotrichomonas* can be plainly seen to be the internal membrane and some such structure must exist in *Metadevescovina*, probably, from their location, the anterior and triangle membranes already described.

In individuals in telophase seen in life (fig. C, 4) a parabasal is often clearly seen wound about each axostyle. When one compares this picture with that of a somewhat earlier stage in which each parabasal is wound about the two axostyles (pl. 10, fig. 9) it seems difficult to picture how the change of position is accomplished.

SYSTEMATIC AFFINITIES

So many of the known forms of the Polymastigina and the Hypermastigina of termites await more critical and complete study and description and so many others are as yet entirely unknown that any attempt at working out their phylogenetic relationships must evidently be but tentative in nature. It is interesting to note, however, how plausible a picture *Metadevescovina* seems to offer of an early step whereby the hypermastigote type may have arisen from the tetramitid type of polymastigote. That *Metadevescovina* furnishes us an example of a much more recent treading of a somewhat similar path rather than a modern survival of an ancient intermediate type, seems probable however, as will be brought out in the discussion of the systematic position of the genus.

In *Metadevescovina* in addition to the tertiary flagella we still have the full set of four of the typical tetramitid even to the trailer which is represented by the primary, whose larger size, posterior position, and relation to the chromatic basal rod and whose peculiar beat all seem to homologize it with the trailer of a *Devescovina.*

Both *Parajoenia* Janicki (1915) and *Caduceia* Franca (1918) *as described* would seem to represent other and more advanced examples of the progressive development of hypermastigote characters in polymastigote stock. Both are in need of reëxamination, however, in the light of our present knowledge of the adherent bacteria and of the neuromotor relations of these organisms.

SYSTEMATIC POSITION OF METADEVESCOVINA

Save for the presence of tertiary flagella, *Metadevescovina* is a typical tetramitid polymastigote. This radical departure from the family type makes necessary the emendation of the family characters or the separation of the new genus in a new family. Three possibilities would present themselves as to the location of such a family. The character which made necessary the removal from the family Tetramitidae would also necessitate the removal of the family to the order Hypermastigina or the erection of an order intermediate between the Polymastigina and the Hypermastigina. The latter would not seem justified. To place a species with such pronounced polymastigote characters among the Hypermastigina seems impossible. There remains therefore to emend the order Polymastigina to allow for the inclusion of the Genus *Metadevescovina* although it seems to represent an early step in the direction of that development of numerous flagella which characterizes the hypermastigotes and to be therefore more or less intermediate between the two orders. Retained in the order Polymastigina it seems more logical to revise the characters of the family Tetramitidae to allow of its inclusion in it with the closely related *Devescovina* than to separate it as a new family on the basis of its tertiary flagella.

That we are dealing here with a recent modification of the tetramitid type and not with a modern persistence of an ancient phylogenetic link seems indicated by the otherwise close resemblance of *Metadevescovina* to specialized tetramitids such as *Devescovina.* This is also indicated by the fact that the corresponding species in the gut fauna of the most closely related termite, *Kalotermes minor* Hagen, is a typical *Devescovina.* Where we have two termites whose morphological similarity is correlated with a similarity in the intestinal faunas we would seem justified in the belief that the termites are phylogenetically related and likewise their protozoan symbiotes. This being true it seems more logical to assume that the common ancestor of the *Devescovina* of *K. minor* and the *Metadevescovina* of *K. hubbardi* was a typical *Devescovina*-like tetramitid and that the tertiaries of *Metadevescovina* have arisen since the differentiation of the two termite species, than to assume that the character was found in the ancestral form and lost in the *Devescovina* of *K. minor* and retained in *Metadevescovina.* In this connection it is interesting, whether significant or not, to note that the more differentiated protozoan is found in the more differentiated termite host.

Aside from the presence of tertiary flagella, *Metadevescovina* differs from *Devescovina* in the peculiar shape and position of its nucleus and in the complicated internal membrane and lamella and the accompanying clear areas. In the absence of the tertiaries these would place it in a separate but closely related genus.

Genus **Metadevescovina** gen. nov.

Diagnosis.—Moderately large, xylophagous polymastigotes similar in most characters to *Devescovina.* They have three approximated secondary (anterior) flagella and a primary (posterior) flagellum in the nature of a trailer. An intracytoplasmic fibril associated with the bases of the secondaries gives rise to a number of short tertiary flagella. A short curved chromatic basal rod underlies the base of the primary.

The nucleus is anterior, transverse, connecting by a neck with the neuromotor center. The axostyle extends throughout the body, ending anteriorly in a loop which integrates with the neuromotor system. The parabasal body is large, long, in a loose left spiral about the nucleus and axostyle. It contains a central thread which integrates it with the primary blepharoplast. Clear spaces are associated with internal membranes in the anterior region of the body.

Type species, *M. debilis* sp. nov., from the hind intestine of *Kalotermes hubbardi* Banks of Arizona and California.

SUMMARY

Metadevescovina debilis gen. nov., sp. nov., from the hind intestine of the termite *Kalotermes hubbardi* Banks is most prominently characterized by the possession, in addition to the ordinary four flagella of the Tetramitidae, of a number of small (tertiary) flagella, arising from an intracytoplasmic fibril. On the basis of number of flagella alone this would place it in the order Hypermastigina. In the number and character of its other flagella and in the other elements of the neuromotor system its affinities are clearly with the tetramitid genus *Devescovina.* The family Tetramitidae and the order Polymastigina must be emended therefore to allow for the inclusion of such forms.

Metadevescovina debilis while intermediate in ordinal characters between the polymastigotes and hypermastigotes does not represent a phylogenetic link between the two orders but merely a tetramitid stock comparatively recently differentiated in the same direction as that probably taken by the ancestors of the hypermastigotes. That this is the case is evidenced by the highly developed tetramitid characters of *M. debilis* and by the fact that the corresponding form in the closely related termite, *K. minor* Hagen, is a typical *Devescovina.*

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EXPLANATION OF PLATE

All figures of *Metadevescovina debilis* gen. nov., sp. nov., from *Kalotermes hubbardi* Banks, based on camera lucida sketches from gut smears fixed in Schaudinn's or Bouin's fluid and stained by the iron haematoxylin method.

PLATE 10

Fig. 1. Anterior end seen from the ventral and left side showing structures of the neuromotor system, the nucleus with karyosomes, the oblique and transverse clear areas and the separate natures of the ventral portion of the transverse membrane and the anterior extension of the axostyle. × 1700.

Fig. 2. Entire animal from left side showing internal structure and coat of attached bacteria except in region of neuromotor center. Secondaries torn loose from the cytoplasm to near their point of origin. × 1700.

Fig. 3. Entire animal from the right side, showing the dense triangle and the relation of its membrane to the parabasal, to the clear areas and to the anterior internal membrane. The spherical structure in the posterior cytosome is a parasite commonly found in this species. × 800.

Fig. 4. A subsurface optical section of the right side of anterior end to show the membrane of the dense triangle in its relation to the parabasal and to the anterior internal membrane. The dark line to the right is the fiber of the anterior margin of the axostylar loop running to the neuromotor center. × 1700.

Fig. 5. Surface view of anterior end of animal somewhat uplifted to show the anterior clear area bordered by margin of anterior internal membrane. × 800.

Fig. 6. Optical section, at level of axostyle, of anterior end of an animal lying on left side, showing relation of axostylar loop to neck of nucleus and of parabasal to groove of ventral angle of membrane of dense triangle. × 1700.

Fig. 7. Surface view of anterior end of an animal lying on left side so elongated and distorted in making smear as to show clearly the continuity of the three clear areas. × 800.

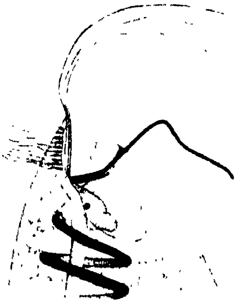
Fig. 8. Somewhat schematic figure of nucleus in prophase with two complete neuromotor sets. Note old exostyle behind nucleus, large, apparently rather loose fibrillar parabasals attached by parabasal threads to the centrosome, while all other neuromotor structures originate from the blepharoplast connected to it by a rhizoplast; deeply staining fibers originating from the blepharoplast and running obliquely downward from it and to the left seem to represent the rudiments of the new axostyles. Note that the one to the right is closely associated with the old axostyle, a condition represented in a much later stage in figure 9. × 2750.

Fig. 9. Entire individual in telophase of mitosis. Note new axostyles, apparently outgrowths, the one on the left attended by remnants of the membrane of the old axostyle. Compare the arrangement of the parabasals with that in a somewhat later stage shown in figure C, 4. × 800.

Fig. 10. Somewhat schematic figure of anaphase nucleus with attached neuromotor structures. The axostyle was not made out in this individual. × 3400.

Fig. 11. Outline sketch of nucleus and neuromotor center from left side to show integrations, particularly the apparent connection of neck of nucleus to inner apex of chromatic basal rod. × 1700.

Fig. 12. Outline sketch of neuromotor structures of anterior end of an animal especially favorable as to stain and position for showing integrations. Note parabasal thread arising from primary blepharoplast while neck of nucleus apparently runs to one of granules at base of secondaries. × 1700.



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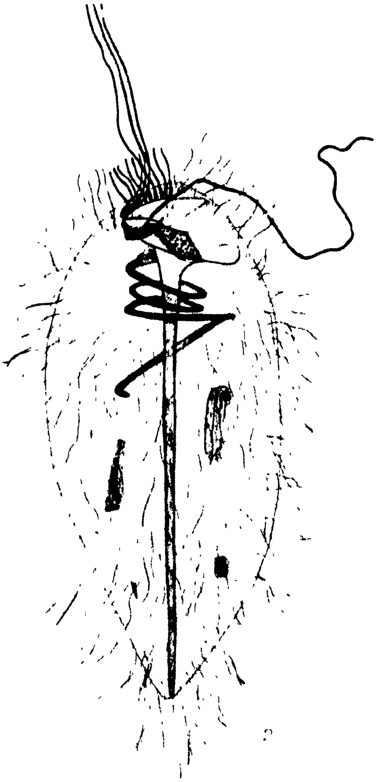

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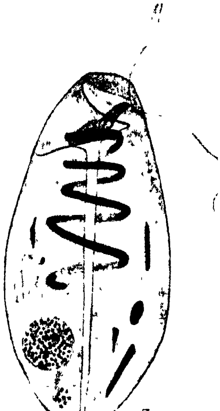

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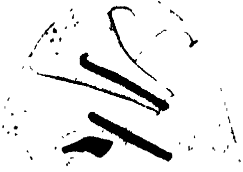

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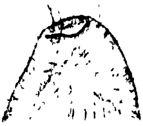

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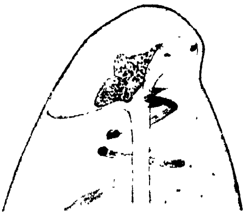

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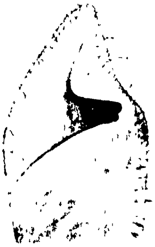

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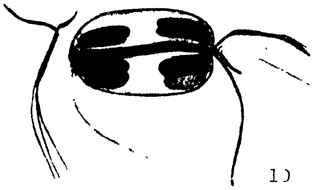

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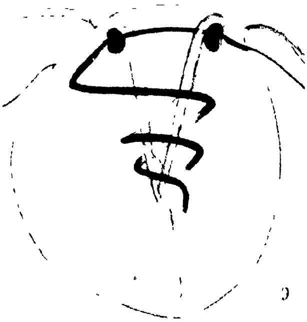

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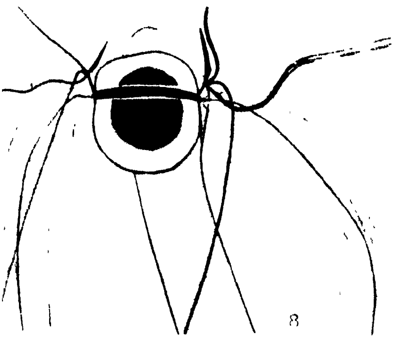

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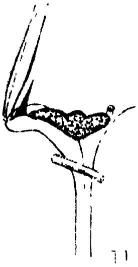

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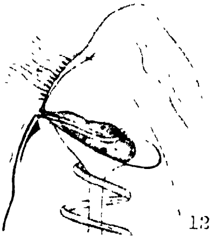

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ON THE MORPHOLOGY AND MITOSIS OF TRICHOMONAS BUCCALIS (GOODEY) KOFOID

BY

H. CORWIN HINSHAW

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INTRODUCTION

Although our knowledge of the morphology of the trichomonad flagellates of lower vertebrates and of laboratory mammals is fairly complete, there is no adequate study of any one of the species living within the human body. The oldest of these, perhaps the least known and certainly one of the most interesting, is *Trichomonas buccalis* of the buccal cavity, which was probably first seen by O. F. Muller in 1773.

The authorship of this species is usually ascribed to Goodey and Wellings (1917). However, the description of the species was contained in a separate paper written by Goodey alone and appended to their joint contribution. According to Article 21, International Rules of Zoological Nomenclature, Paris, 1905, the authority for this species should be Goodey. The species was transferred from the genus *Tetratrichomonas* to *Trichomonas* by Kofoid (1920).

ACKNOWLEDGMENTS

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MATERIAL AND METHODS

The present work is the by-product of an extensive survey of the parasitology of pyorrhea alveolaris. Approximately 300 normal and pathological mouths have been examined by the cultural method to date. We have secured 69 primary cultures of *Trichomonas buccalis* from the 53 persons found to be infected. A detailed report of the incidence of the protozoan parasites of the human mouth will appear in a later communication. Suffice it to say here that *Trichomonas buccalis* has thus far been found by us only in cases of advanced and active pyorrhea.

THE CYTOPLASM

The cytoplasm may vary in appearance, depending upon the fixing fluid employed, the age of the culture, etc. Young, vigorous cultures fixed well in hot Schaudinn's fluid usually yield specimens with finely granular and indistinctly alveolar cytoplasm. The clear alveoli are often small in size, irregular in shape, and may escape detection upon superficial observation. Occasionally individuals are met with possessing large clear spherical vacuoles, more commonly located in the posterior region. Such specimens are more common in older cultures. Likewise, forms with many stainable granules in the cytoplasm are more commonly met with in senescent cultures. These granules are seldom as conspicuous as figured by other observers in related species.

Few solid food particles seem to be ingested by *Trichomonas buccalis* in cultures. Although it is common to see a few cocci in food vacuoles, these constitute a very slight bulk.

The existence of a cytostome is not certain in all specimens, but an indefinite clear area, triangular in shape, which we regard as the cytostome, is frequently seen near the origin of the undulating membrane. The latter borders this cytostome on one side and probably serves to waft food materials into the gullet. We shall speak of this surface which bears the undulating membrane and cytostome as the ventral surface.

There is a pellicle surrounding the entire body. It appears to be thicker over the posterior two-thirds of the body. The more thinly covered anterior third is the region subject to greater mobility in life.

THE NEUROMOTOR SYSTEM

The blepharoplast is not a single body as it is figured by other observers, nor is it double in adult forms as Hogue (1926) describes it. It consists of a primary central granule (fig. A, *prim. cent. bleph.*) giving rise to the two central flagella, a secondary ventral granule (fig. A, *sec. vent. bleph.*) giving rise to the undulating membrane, chromatic basal rod, and the independent ventral flagellum, and a secondary dorsal granule (fig. A, *sec. dors, bleph.*) giving rise to the axostyle and the dorsal flagellum. Frequently these granules are

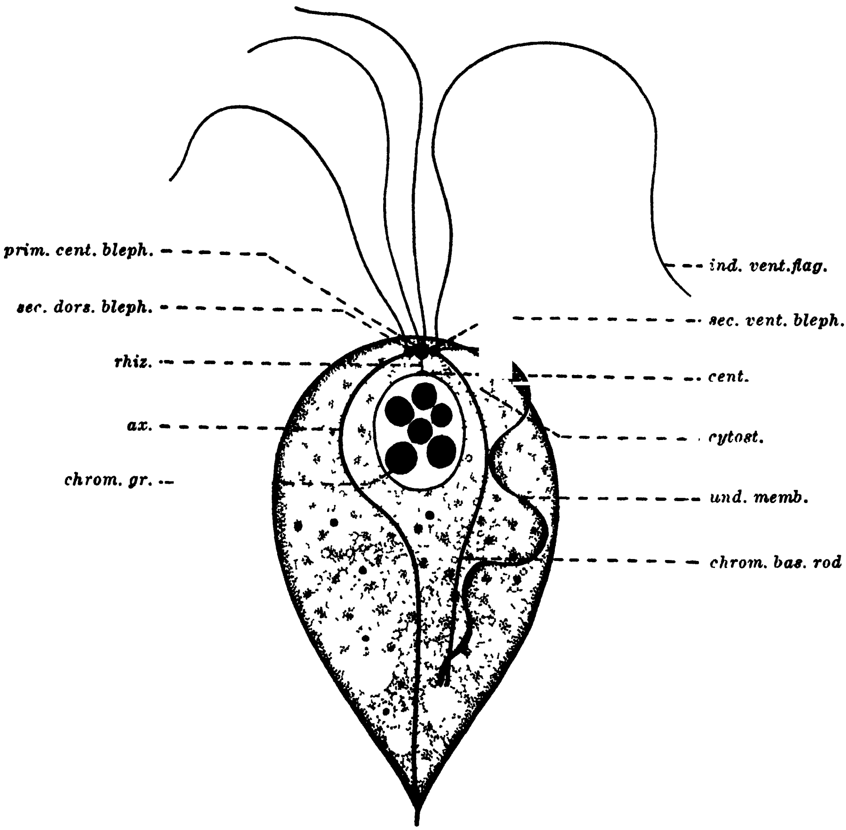

Fig. A. Diagrammatic figure of right side of *Trichomonas buccalis* (Goodey) Kofoid. × 6800. From stained preparation in culture. *ax.*, axostyle; *cent.*, centrosome; *chrom. bas. rod,* chromatic basal rod; *chrom. gr.*, chromatin granules; *cytost.*, cytostome; *ind. vent. flag.*, independent ventral flagellum; *prim. cent. bleph.*, primary central blepharoplast; *rhiz.*, rhizoplast; *sec. dors. bleph.*, secondary dorsal blepharoplast; *sec. vent. bleph.*, secondary ventral blepharoplast; *und. memb.*, undulating membrane.

more or less closely grouped (pl. 11, fig. 2). It is only in favorable specimens that the true nature of the blepharoplast can be determined. More than three granules are never present and these always bear the same relation to other parts of the neuromotor system. In young individuals without an axostyle there are only two granules (pl. 11, fig. 7).

The position of the centrosome cannot be accurately determined in most individuals in the interphase. The rhizoplast connecting the blepharoplast with the nucleus may appear to terminate in a very small, irregular, siderophile granule lying on the nuclear membrane which we regard as the centrosome. The centrosome is quite clear during binary fission as will be shown later.

The flagella lie at the anterior tip of the body and are four in number. Three of these are of approximately equal length in adult specimens; the dorsal flagellum and the two central flagella (fig. A). The independent ventral flagellum may lie apart from the others and is frequently one or two microns greater in length (pl. 11, fig. 1). When the flagella are entangled or coiled it is obviously difficult to detect this difference in length. Consequently Hogue (1926) considers that the flagella are of equal length.

Young individuals may possess only three flagella for a short time after fission, the dorsal flagellum being last to appear since it is connected so intimately with the axostyle, which organelle is the last to be regenerated following fission (pl. 11, fig. 8). At a later stage one may find two long and two shorter flagella, a condition which Goodey (1917) considered to be characteristic of the species in the adult phase.

In life the undulating membrane is frequently very conspicuous, but in stained specimens that are well differentiated it is often obscure except for the marginal filament.

In life it is impossible to see a short posterior flagellum formed of the free marginal filament such as some other trichomonads exhibit. In fixed material a very short posterior flagellum may be present but it is probably due to artificial detachment. When alcoholic eosin is added to the living culture this marginal filament becomes almost completely detached in a large percentage of the animals.

The membrane follows a slightly dexiotropic spiral course, extending posteriorly from two-thirds to three-fourths the length of the body. It is not as long as in other members of the genus.

The chromatic basal rod is uniformly present in well fixed and stained specimens but has frequently escaped detection (Ohira and Noguchi, 1917; Hogue, 1926). Only the most careful technique brings out its chromatic nature to the best advantage. It consists of a slender curved siderophile rod in the cytoplasm adjacent to the undulating membrane. Sometimes it exceeds the undulating membrane in length. It arises from the same granule of the blepharoplast that gives rise to the ventral flagellum and the undulating membrane. Occasionally it lies in a wavy line (pl. 11, fig. 1) indicating possibly that in life its undulations correspond to the movements of the undulating membrane with which it is so closely associated.

The axostyle consists of a slender siderophile rod arising from the dorsal secondary blepharoplast, curving around the nucleus, and running in the median line to the posterior tip of the body from which it may project a short distance. It always lies on the side of the nucleus opposite the chromatic basal rod and the undulating membrane.

THE NUCLEUS

The nucleus is a round or oval body lying in the anterior one-third of the body. It is surrounded by the usually distinct nuclear membrane which encloses a clear area containing six or more deeply stained granules. When only six granules are present, one is led to suspect that they represent the three chromosomes in a precocious split condition. Not uncommonly, however, there are many granules of varying sizes present (pl. 11, fig. 10). These granules are usually distinct in outline, varying in shape from spherical to ovoid. They stain intensely with the iron-haematoxylin method, retaining the stain long after the remainder of the organism is colorless. To display the nucleus to best advantage, it is often necessary to destain the specimen beyond the optimum point for the observation of cytoplasmic structures. This great affinity of the nucleus for chromatic dyes has led to the report of previous observers (Goodey, 1917; Hogue, 1926) that the nucleus is commonly homogeneously black, usually possessing no internal structure. Correct destaining of properly fixed individuals never fails to reveal the internal structure of the nucleus, as has been described above. The erroneous reports of the above named investigators must be ascribed to faulty technique. Specimens allowed to dry before fixation or specimens fixed in defective fluid may show this same uniform staining of the nucleus, which is in no case a true picture of existing structure.

Individuals showing the nucleus with a single central karyosome connected by spoke radii to a peripheral ring of chromatin are occasionally found but are very rare in our cultures.

THE LIVING ANIMAL

In life *Trichomonas buccalis* has impressed all observers with its remarkable activity, being more agile than many other species. The anterior one-third of the body is very plastic and protean.

The locomotion of this organism is chiefly effected by the concerted stroke of the three shorter powerful anterior flagella. While these flagella are being stretched out in their forward stroke, the anterior tip of the body forms a cone-shaped projection of the protoplasm of their base. With the powerful backward stroke this cone is rapidly retracted, giving an added impetus to the flagella resembling the common trick of 'cracking the whip.' This process is repeated with every stroke, occurring several times in the course of a single second. The line of locomotion is always in a spiral, there being approximately one revolution of the body while it moves through a distance equal to its own length.

In actively motile or in moribund individuals it is difficult to observe a differential behavior of the flagella. However, if a sealed slide is placed at a temperature near 0° C. for 24 hours and is quickly examined during the period when the organisms are beginning to resume flagellar activity, it is frequently possible to observe that the independent ventral flagellum behaves differently from the others. As might be expected from its neuromotor connections, it beats in rhythm with the undulating membrane and is first to be endowed with movement during the process of warming. Its stroke is more laterally directed than the others and its tip describes a flattened circle serving to aid the undulating membrane as a rotator of the body. In behavior, in length, and in neuromotor connections it is closely comparable to the independent anterior flagellum of *Pentatrichomonas* (Kofoid and Swezy, 1923), with which we consider it to be homologous.

The marginal filament of the undulating membrane does not move laterally in a single plane but describes a coiled right-hand or dexiotropic and somewhat flattened spiral in motion.

If one watches a single organism continuously for several minutes, it is frequently observed to undergo a cycle of activity. It will actively swim about for a time, shortly attaching itself by the posterior tip of the body while the anterior flagella create a veritable maelstrom by their rapid vibratory strokes, passing in rapid review the material of the environment. Tiring of this activity, the organism will seek shelter within a clump of mucoid débris, forcing its way through small interstices by protean activity of the entire body. Often the flagella will seek a firm hold in a more solid region, seeming to draw the body forward by a relatively slow but plainly powerful flexing motion. In serving this function the flagella appear almost to possess the power of prehension, and one wonders at the strength resident in these slender strands of protoplasm. In this reaction there is plainly a strong stereotropic impulse at play. Once in sufficiently intimate contact with a semi-solid environment the trichomonad comes to a state of relative quiescence, with flagella lying at rest directed laterally or slightly posteriorly. The slow undulations of the undulating membrane are the only evidences of activity, save an occasional feeble stroke of the flagella. Such an individual is usually assumed to be moribund but if it is displaced from its shelter it resumes its activity with undiminished vigor. Or if watched for a sufficient period of time it will, often without visible stimulus, leave this retreat for active life in the more open regions. These observations may have a bearing on the activities of the organism in its normal habitat, which will be discussed later.

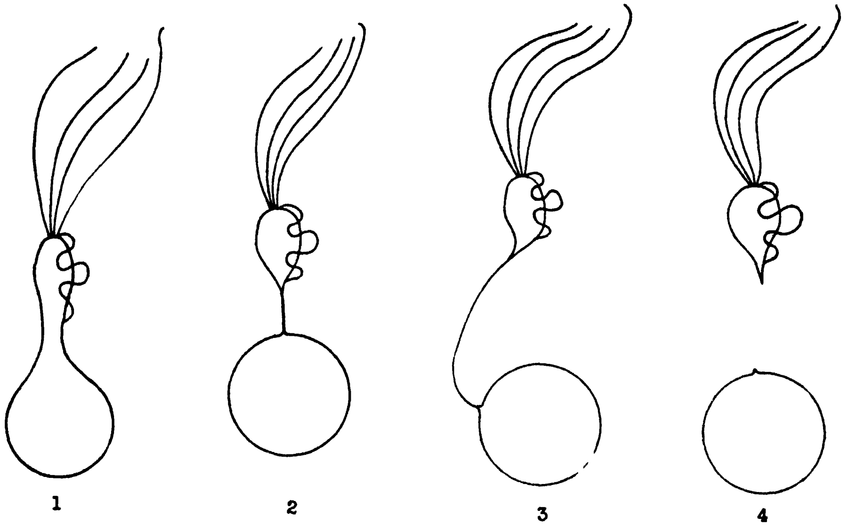

Fig. B. Outline sketches of *Trichomonas buccalis* (Goodey) Kofoid undergoing a process of autotomy. × 3000.

Some interesting observations of a radical process of autotomy have been made on animals in a toxic environment. Figure B consists of a series of camera lucida drawings of a living animal undergoing this process. The repeated protrusion and retraction of a club-shaped anterior proboscis marks the period prior to this process of self-pruning (fig. B 1). Some organisms cease with this and within the period of observation do not complete the act of autotomy. Others may proceed to constrict the neck region of this flask-shaped body, resulting in complete separation of the parts save for a slender strand of protoplasm (fig. B 2), which may be drawn out to a surprising length (fig. B 3). Finally this connection is broken, the strand quickly disappears, and the dwarfed *Trichomonas* bearing the entire undulating membrane, the nucleus, and neuromotor system, including the long flagella, swims away (fig. B 4). It is in no sense a normal thing for such marked autotomy to occur although a similar but less drastic habit has been observed (Kofoid and Swezy, 1915) with evident intent at reduction of body volume. Stained specimens in our own preparations also exhibit this phenomenon, especially in senescent cultures. It is probable that this is one method of excretion, a theory which is supported by the fact that in a toxic solution, such as urethane, a number of individuals have been observed by us to cut off the major part of the cytoplasm in an effort to cast out the injurious substance. This is not unlike the method of holocrine secretion as it occurs in the alimentary canal of insects.

BINARY FISSION

The earliest indication of nuclear division is the arrangement of the granules into three rows, as represented in figure 2, plate 11. A condensation into three distinct chromosomes next occurs (pl. 11, fig. 3). Each chromosome is of distinctive shape and size. The first is somewhat fusiform in shape and tends to lie in a straight line. The second chromosome is similar in size but is bent into the form of a crescent (pl. 11, figs. 3–6). The third chromosome uniformly possesses a very large asymmetrical club-shaped end. At the opposite end there may be a smaller enlargement which is frequently either hidden or absent when the chromosomes are grouped into the equatorial plate (pl. 11, figs. 4–6). It is evident that the shape of chromosomes is not determined by their spindle fiber attachments, since they are readily recognizable in this prophase.

It is evident that the grouping of chromosomes into the equatorial plate takes place very quickly following their formation for no intermediate stages have been observed. The equatorial grouping is retained for a considerable length of time, however, for such stages are relatively common in the actively growing cultures. The splitting of the chromosomes and their migration to the poles must take place with great rapidity, for prolonged search has failed to reveal such stages in our preparations.

It is entirely possible that the six granules, sometimes seen in the resting nucleus, represent the three chromosomes in a precocious split. In the prophase these 'daughter chromosomes' may unite and fuse (pl. 11, fig. 2) end to end, parting again in the metaphase (pl. 11, fig. 6). To see only the latter phase of this process would give the false impression that the true split occurred in the transverse plane.

The telophase stage as represented in figure 9, plate 11, is one of the most frequently observed of all division stages. The daughter nuclei have now become exceedingly dense, often showing their triplex nature, with three lobes representing the three chromosomes in a condensed state. A heavy extranuclear siderophile strand, the paradesmose (Kofoid and Swezy, 1915), is a striking feature of this stage.

The centrosome evidently divides before the blepharoplast. In figure 4, plate 11, is seen an individual the centrosome of which has divided, the daughter centrosomes being separated by a paradesmose lying above the nucleus and each being connected to the condensed, dividing blepharoplast by a very delicate rhizoplast. The chromatic basal rod lies under the nucleus, being scarcely visible in this specimen. The axostyle retains its integrity but shows signs of degeneration by loss of stainability. The four flagella are evident, the undulating membrane is not clear but is evidently not duplicated as yet.

While the chromosomes are closely clumped in the equatorial plate (pl. 11, figs. 5–6), the centrosomes lie immediately outside the distinct nuclear membrane. They are connected by a conspicuous paradesmose lying above the chromosomes and outside the nuclear membrane. At this stage one daughter blepharoplast may be observed to retain the old undulating membrane and chromatic basal rod for a short time at least, while the other daughter blepharoplast immediately starts the outgrowth of new organelles. The flagella are evenly divided between the poles, two going to each.

Distinct spindle fibers are sometimes seen (pl. 11, fig. 6). In this individual the crescent-shaped chromosome is undergoing an apparent transverse constriction, which may or may not be the end of its metaphase split.

During the telophase (pl. 11, figs. 7, 9) the undulating membrane is duplicated together with the chromatic basal rod. Still there are but two flagella to each pole, duplication by new outgrowth evidently taking place after plasmotomy in rapidly dividing individuals.

Even the axostyle may not be regenerated before plasmotomy occurs (pl. 11, fig. 7). In such individuals the paradesmose has sufficient resemblance to an axostyle to cause confusion. This point is amplified and discussed in a later paragraph.

Ohira and Noguchi (1917) have reported mitotic division of the blepharoplast. Their single figure on this point shows plainly a malformed nuclear spindle, and there is no conceivable reason for considering this to be a blepharoplast.

DISCUSSION

The origin of new axostyles in daughter individuals has been variously interpreted by different investigators. In *Tritrichomonas augusta* and in *Tritrichomonas muris*, Kofoid and Swezy (1915) describe a longitudinal splitting of the parental axostyle as does Wenyon (1907). Other observers have reported the resorption of the old axostyle and the regeneration of new axostyles from the daughter blepharoplasts.

Dobell (1909), working on both *Trichomonas batrachorum* and *Trichomastix batrachorum* reports resorption of the old axostyle and the formation of new axostyles from the thread (paradesmose of Kofoid and Swezy, 1915) connecting daughter individuals. MacKinnon (1910) reported that in the division of *Trichomonas trichopterae* the old axostyle disappears and the new axostyles are derived from the thread connecting the divided basal granules. Alexeieff (1910) states that the same is true of *Trichomastix motellae*. Janicki (1910) publishes figures to demonstrate that a similar origin of the new axostyles occurs in *Lophomonas blattarum*. Jollos (1911) considers that the new axostyles of *Monocercomonas cetoniae* arise in a similar manner.

With this mass of testimony and the evidence of such specimens as illustrated in figure 7, plate 11, the preceding explanation would appear plausible. In this specimen the paradesmose connecting the daughter individuals resembles a common axostyle very closely in position, in neuromotor connections, and in staining reaction. And although the daughter chromatic basal rods are present, there is little evidence of any axostyle other than the paradesmose. Persistent search has brought to light such forms as figure 8, plate 11, which certainly constitute decisive evidence that the daughter axostyles arise *de novo*, and in some cases after plasmotomy. In the individual figured, the portion of paradesmose is undergoing resorption with a distal granular degeneration, while the true axostyle and chromatic basal rod are unmistakably present at the same time, leaving no ground for a belief that the axostyles are either derived from the paradesmose (Dobell, 1909) or by a splitting of the axostyle (Kofoid and Swezy, 1915).

In cultures where conditions are nicely controlled we have observed that rapidly growing, vigorous cultures exhibit a type of fission quite distinct from that occurring in senescent cultures. In the former, growth, nuclear division, and plasmotomy occur in rapid succession, as described in the preceding section. The regeneration of neuromotor organelles takes place more slowly. Daughters may be freed without an axostyle, with only two flagella, and with a compact nucleus such as characterizes the telophase. In senescent cultures plasmotomy is delayed. The paradesmose disintegrates, the axostyles are regenerated, and the nuclei are broken up into granules, before plasmotomy occurs. Such duplex individuals (pl. 11, fig. 10) might be erroneously taken as fused isogamous conjugants if the sequence of stages be reversed in order.

The life cycle of *Copromonas subtilis* as described by Dobell (1908) bears a strong superficial resemblance, at least, to the dual cycles just described for *Trichomonas buccalis*. In young vigorous cultures he described typical fission, involving the formation of a conspicuous persistent chromatic thread (paradesmose of Kofoid and Swezy, 1915) such as we report for *Trichomonas buccalis*. He stated that in senescent cultures duplicate individuals occurred, which were considered to arise by the fusion of isogamous gametic individuals. The figures of stained 'conjugating' individuals of *Copromonas subtilis* published by Dobell bear a striking resemblance to what we regard as delayed fission in senescent cultures of *Trichomonas buccalis*. In the absence of any cytological evidence of conjugation in either of these forms we refrain from speaking of sexual reproduction in *Copromonas* or in *Trichomonas*.

From their extensive observations on the trichomonad flagellates of lower animals, Kofoid and Swezy (1915) conclude that the axostyle is to be considered as homologous with an intracytoplasmic flagellum. Their principal difficulty with stressing this homology was due to the form and staining reactions of the axostyle in their material. In most trichomonads the axostyle is a thick hyaline rod, bearing little morphological resemblance to a flagellum except in its neuromotor connections. The conclusion reached by Kofoid and Swezy was based chiefly on the locomotor function which they observed this organ to possess. In *Trichomonas buccalis* we have some interesting morphological observations which tend strongly to confirm the homology suggested by Kofoid and Swezy. Obviously in *Trichomonas buccalis* the axostyle is more primitive, for it resembles in every respect an intracytoplasmic flagellum. It is siderophile, unlike the hyaline rod in many other members of the genus. It is slender, its diameter not greatly exceeding that of the extracytoplasmic flagella. It is connected with the dorsal blepharoplast which also gives rise to one extracytoplasmic flagellum, the central blepharoplast giving rise to two flagella, while the ventral blepharoplast gives origin to one flagellum and the marginal filament of the undulating membrane, which latter has undoubted flagellar homologies. Thus each of the three blepharoplasts gives origin to two flagella if we consider the axostyle as an intracytoplasmic flagellum. This homology would thus tend to be established by a process of reasoning similar to that employed in determining serial homology in metameric animals. Furthermore, in its method of regeneration by outgrowth following binary fission, it resembles the other flagella.

The varied functions of the undulating membrane have remained uncertain to date. Obviously it serves to a certain extent in locomotion, chiefly as a rotator to give a spiral motion to the organism in progression, since it is observed to run a slightly diagonal course across the body. Locomotion may not be as dominant a function in the life of these and many other animals as microscopic observation would lead us to believe. When under microscopic scrutiny their environment is far from normal. Light is irritating to many forms of protoplasm and the longer wave lengths are absent from the normal habitat of most parasitic protozoa. Under the microscope the extremely concentrated light may irritate the organism to an abnormal state of activity. As stated in a previous section, continuous observation of a single organism will show a perfectly normal tendency of the organism to bury itself in solid material and cease active locomotion. In this state the flagella are inactive, but the undulating membrane never ceases to bathe the body with fresh fluid every instant. In this, which is probably the organism's most normal attitude, the undulating membrane comes into an indispensable rôle. It serves to make possible all interchange which takes place between the animal and its environment, including respiration, excretion, and nutrition. In observing this, one is led to compare the undulating membrane to some accessory respiratory organs of higher invertebrates, such as the maxillae of crustacea.

Calkins (1926) would limit the term 'chromosomes' to "compact intranuclear aggregates of chromomeres which divide as unit structures and which are resolved into chromomeres after such division." He states that in the Protozoa "we have absolutely no evidence of qualitative differences and no evidence of individuality" of chromosomes. It is true that in many Protozoa the chromosomes are morphologically similar to each other, but the same is equally true of many Metazoan cells. However, *Trichomonas buccalis* fulfills Calkins' dicta almost perfectly, rigid as they are. Each of the three chromosomes is readily distinguishable from the other two. The morphological distinctions are here most striking. Each chromosome is formed by a linear aggregation of granules (chromomeres) (pl. 11, figs. 2–3). There is a parallel grouping in the equatorial plate at which time definite spindle fibers appear. Our observations have not entirely covered the matter of the splitting of the chromosomes, but we have no reason for believing that it is fundamentally different from the process occurring in metazoan cells or from that observed by Kofoid and his collaborators and students in a wide variety of Protozoa. In short we do not believe that there is any fundamental distinction between cellular division in the Metazoa and in the Protozoa. Whenever refined cytological methods and persistent search of adequate material have been applied, the same sequence of events has been observed in protozoan cells as in metazoan cells.

The existence of an odd number of chromosomes, each distinct morphologically from the others, is evidence that this species leads a haploid life, as indeed might be expected if we consider the holozoic flagellates as the most primitive of Protozoa with no convincing account of sexual reproduction within their ranks. The haploid condition is logically the primitive chromosome complex, the diploid condition appearing in the phylogenetic scale only after sexual reproduction appears (Kofoid, 1923).

SUMMARY

1. *Trichomonas buccalis* possesses the organs characteristic of the genus. The nucleus never stains solid black with proper technique. The blepharoplast consists of dorsal, central, and ventral granules. The chromatic basal rod is invariably present. The axostyle is siderophile and homologous with an intracytoplasmic flagellum.

2. Radical autotomy can be experimentally induced by treatment with urethane and probably serves the function of excretion.

3. While ordinarily intensely active in very fluid material, *Trichomonas buccalis* is relatively inactive in a more normal habitat.

4. Three readily recognizable, structurally different chromosomes appear at mitosis. *Trichomonas buccalis* is in a haploid state.

5. A heavy paradesmose connects daughter centrosomes and disappears following plasmotomy.

6. The flagella are evenly divided between the two daughters. The full complement may not be restored by new outgrowth until after plasmotomy.

7. The old axostyle degenerates and new daughter axostyles grow out from the blepharoplasts after plasmotomy.

8. In senescent cultures plasmotomy is delayed, resulting in duplex individuals resembling conjugated forms. Similar observations on other forms have led to erroneous reports of sexual reproduction.

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EXPLANATION OF PLATE

All figures are made with camera lucida from smears of cultures fixed in hot Schaudinn's fluid and stained with iron-haematoxylin. × 3200.

PLATE 11

Trichomonas buccalis

Fig. 1. Typical form showing three blepharoplasts clearly. Note the wavy appearance of the axostyle and the chromatic basal rod. Note greater length and isolated position of independent ventral flagellum.

Fig. 2. Early prophase. Note the linear arrangement of chromatin granules, later to form chromosomes.

Fig. 3. Early prophase, showing further condensation of chromatin granules into chromosomes. The crescent-shaped chromosome is recognizable.

Fig. 4. Prophase showing fully formed chromosomes. The centrosome has divided, the daughter centrosomes are connected to each other by the paradesmose and with the blepharoplast by delicate rhizoplasts.

Fig. 5. Equatorial plate. The blepharoplast has divided, each daughter taking two flagella. The axostyle has degenerated. The centrosomes lie in contact with the nuclear membrane which is quite intact and distinct.

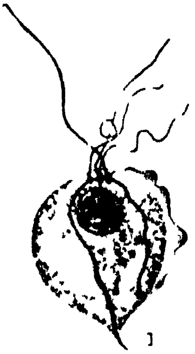
Fig. 6. Equatorial plate. Spindle fibers are distinct. The crescent-shaped chromosome is evidently splitting.

Fig. 7. Plasmotomy. Daughter individuals are connected only by the paradesmose which resembles a common axostyle. The axostyles have not yet been regenerated.

Fig. 8. Young specimen, as indicated by compact nucleus. Axostyle and chromatic basal rod are present, together with the degenerating fragment of paradesmose. The dorsal flagellum has not yet grown out.

Fig. 9. Telophase. Note heavy paradesmose. The body is elongating preparatory to plasmotomy. Each nucleus has a short undulating membrane and a shorter chromatic basal rod with no axostyle.

Fig. 10. Duplex individual with two complete sets of organelles, except for the fact that flagella are not yet regenerated in the daughters. There is no conspicuous paradesmose since division has occurred long ago. From culture eleven days old.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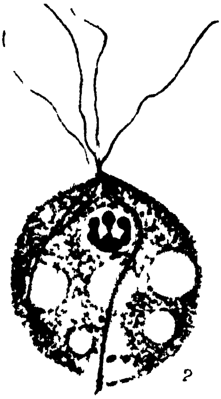
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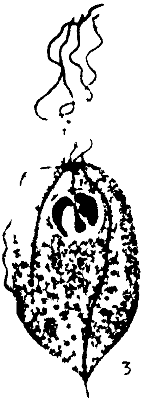
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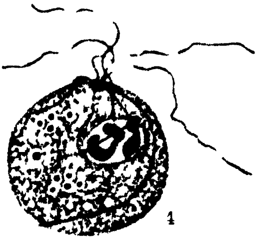
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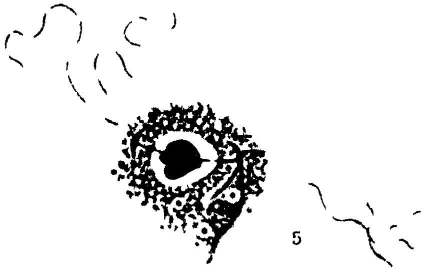
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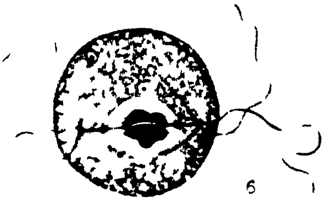
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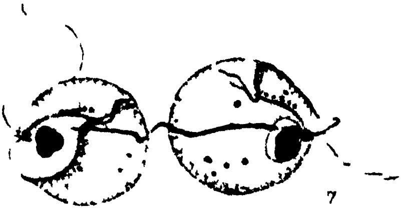
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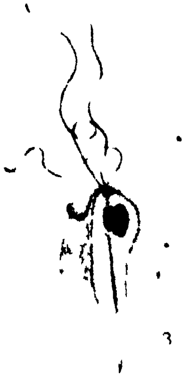
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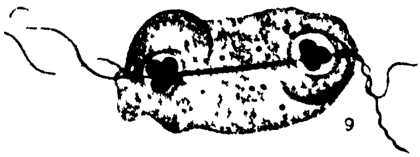
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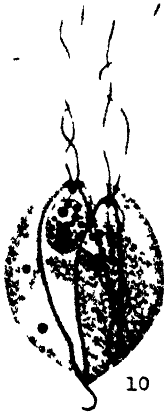
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EXCYSTMENT OF COUNCILMANIA LAFLEURI KOFOID AND SWEZY IN CULTURE IN VITRO

BY

ENA A. ALLEN

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BY

ENA A. ALLEN

According to Kofoid and Swezy (1921) and Kofoid, Swezy, and Kessel (1924), the genus *Councilmania* is separated from *Endamoeba* by differences in nuclear structure and because of the fact that cysts of the former reproduce by budding in the bowel while cysts of no species of *Endamoeba* have been known to bud therein. Wenyon (1925) and Gunn (1922) have suggested that the excystment by budding of amoebulae described for *Councilmania* is an artifact due to pressure, although Kofoid and Swezy (1921) state that it is found in sections of fixed stool treated without pressure. The notes here recorded deal with this normal process of excystment as observed.

It is with sincere gratitude that the writer acknowledges her indebtedness to Dr. C. A. Kofoid, who has given valuable assistance and who has generously granted the privileges of his laboratory and his private library.

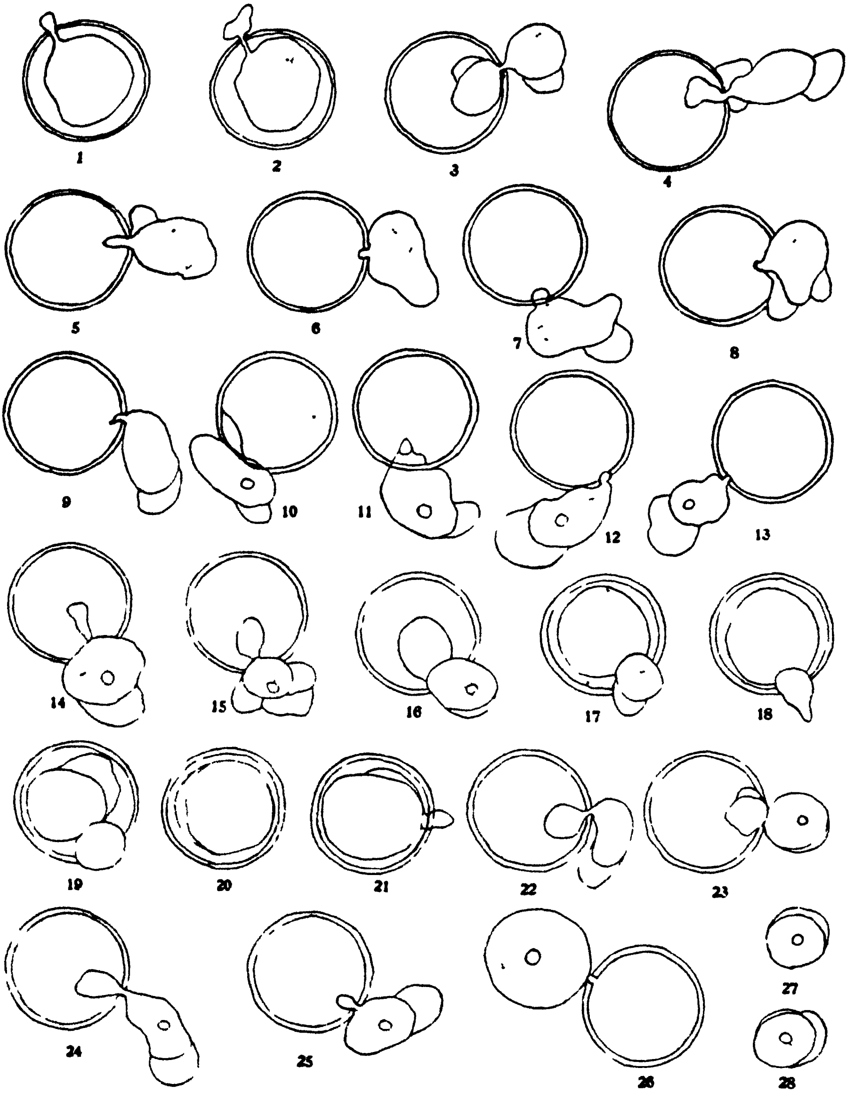
A specimen containing very few motile forms and many cysts, some of which were observed to be budding in fresh smears without other pressure than that of the floating coverslip, was obtained from a case positive for *Councilmania lafleuri*. This case has been diagnosed fifty-two times over a period of four years in the Protozoological Laboratory of the University of California and, in addition to this, forty-four specimens have been obtained for culture experiments. All the ninety-six stools were positive for *Councilmania lafleuri*. Some were positive also for *Endamoeba coli*, but the latter has not been found in this patient since July, 1925.

Because of the presence of *Blastocystis hominis* various media were used in an attempt to get some cultures started. It was hoped that the few motile forms would multiply and that the budding cysts would excyst. Among the media used was Ringer's solution plus .01 per cent dextrine. Drbohlav (1925) by chance discovered that the addition of one per cent dextrine to the Ringer's solution, for two or three transplants, destroys *Blastocystis hominis*.

Forty culture tubes, sixteen of which contained Ringer's solution plus .01 per cent dextrine (without egg slants), were inoculated from the specimen mentioned above. In subcultures from three of the sixteen tubes excystment took place. This was evidently begun in the intestine of the patient and was entirely completed within forty-eight hours after the first tubes were inoculated.

In sediment from two of the three subcultures some amoebulae and some empty cysts, showing the typical pore, were seen; but in sediment from the third tube one instance of the final act of excystment was under observation for forty minutes. The cyst observed was a normal one in all respects with reduced protoplasmic content suggestive of the results of progressive excystment of amoebulae prior to observation. Observations upon excystment in the cytoplasm remaining within the cyst wall showed only one nucleus which was clearly visible. There are usually eight, sometimes sixteen, nuclei in a mature cyst of *Councilmania lafleuri,* so the other seven had presumably already escaped in amoebulae budded off from the original protoplasm. Very near, within the radius of the microscopic field, were two smaller amoebulae (figs. 27 and 28) which had probably budded from this cyst since no other cysts could be found on the slide. The one still within the parent wall moved actively about and when first observed had begun to crawl through the pore from which the other amoebulae had escaped during the budding process. It continually sent out clear pseudopodia, with the surprising suddenness so characteristic of *Councilmania lafleuri,* while it was flowing out through the pore. When nearly out it suddenly began to go back and returned entirely within the cyst wall again. It rounded up for a few moments, then became very active and by degrees extricated itself from the old cyst wall and rounded up in the medium about the cyst. At this time the single nucleus was again distinctly visible. The freedom from pressure on the cyst wall was indicated by the way it bobbed about each time a new pseudopod was formed by the amoebula when it was passing through the pore and especially when it was just barely hanging at the outside edge of the pore.

It is interesting to note that this last young amoeba was considerably larger than the two other amoebulae seen on this slide. This would indicate one of two things: either that the larger part of the original cytoplasm was left for the eighth individual, or that the latter had increased in size after the others budded off. No doubt liquid media and bacteria could enter through the pore. It is possible,



Figs. 1–28. Excystment of *Councilmania lafleuri*

These drawings were made free-hand during observations on excystment, at a magnification of about 900.

Figs. 1–26 inclusive represent successive stages of the final act of excystment of the last amoebula from a single cyst. Note the change in position of the pore which indicates the freedom with which the emerging amoeba moved about the cyst in absence of any confining pressure. Observe especially figures 9 and 10 at which time the amoeba and cyst turned, reversing the position of the pore.

Fig. 20. Shows the amoeba retrated entirely within the cyst wall again.

Fig. 26. Amoeba completely outside of cyst wall in which the pore is clearly visible.

Figs. 10–16, and 23–26 show the nucleus, which was very distinctly seen in these positions of the amoeba.

Figs. 27 and 28 are two amoebulae which were within the diameter of the microscopic field near the excysting amoeba.

also, that this last amoebula, because of plenty of room, remained within the parent wall for some time after all the others had crept out.

It is evident from this observation that excystment depends upon a number of things. First of all, cysts must be ripe, that is, just ready to bud when put into a medium suited for excystment, or a medium is required which will stimulate a cyst to reach maturity and then to bud. External factors, as well as internal conditions of the cyst, must be considered since they are of equal importance. Chemical stimuli, temperature, and osmotic pressure all have their part to perform in this as well as in other culture experiments. The small percentage of amoebae which were known to excyst was not only due to proper conditions but also to the fact that allowance was made for immature and for moribund cysts.

SUMMARY

Excystment of *Councilmania lafleuri* took place in Ringer's solution plus .01 per cent of dextrine only, and not in any of the other media used. Of the sixteen tubes of Ringer's solution and dextrine only three (a little more than 20 per cent) were found to contain excysting amoebae.

Cysts must reach the proper phase in their life-history in order that excystment can take place and the surrounding medium must have within it the necessary chemical and physical conditions to make possible this excystment.

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THE
CYTOLOGY OF TINTINNOPSIS NUCULA (FOL) LAACKMANN

WITH AN ACCOUNT OF ITS NEUROMOTOR APPARATUS, DIVISION, AND A NEW INTRANUCLEAR PARASITE

BY

ARTHUR SHACKLETON CAMPBELL

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INTRODUCTION

Cytological evidences presented by a study of *Tintinnopsis* offered in this paper may be of advantage to further investigations in revealing in a striking manner great structural differentiation and integrated behavior in this interesting, neglected, and large group of primarily marine, house-building ciliates. Particular attention is devoted to the neuromotor system, described for the first time for any of the tintinnids, and to the nuclear and cytoplasmic phenomena at the critical period of division. The relation of the house or shell to the neuromotor system and the method of its origin is discussed.

ACKNOWLEDGMENTS

The writer takes this occasion to express his indebtedness to Dr. C. A. Kofoid, at whose instance the investigation was undertaken, for kindly and suggestive aid. The Scripps Institution of Oceanography, of La Jolla, through its Director, Dr. T. Wayland Vaughan, has rendered invaluable aid, not only in allowing the writer the generous use of ample facilities during the several weeks of the summer of 1925 which he spent there, but also in supplying an abundance of well prepared material during the greater part of the year.

TECHNIQUE

Collections with a number 25 silk bolting cloth net were made by the writer at various periods throughout the year, depending upon tides and bright days. Most of the material used was collected at the Key System Railway pier, Oakland, California. The incoming tide early in the morning of a bright sunny spring day proved to be the most productive of abundant material. During the summer, the writer collected material at the Scripps Institution of La Jolla, California, under somewhat similar conditions.

As soon as containers were brought into the laboratory the contents were filtered quickly so that the excess amount of sea water might be eliminated. The precipitate, consisting of the organisms of the catch remaining upon the filter paper, was washed quickly and carefully into a large, shallow crystallization dish and allowed to remain at least one-half hour in a little sea water before being killed.

It was found after considerable experimentation that by this simple method the majority of the tintinnids, as well as other plankton, could be killed in an expanded state. Contracted animals are useless. The killing agent was always used hot, and if poured in suddenly with a rapid rotary motion nearly every animal in the crystallization dish was killed while excellently expanded.

Various fixing reagents were used. None proved satisfactory but Schaudinn's alcohol-mercuric chloride-acetic acid mixture, and this only when heated to boiling. Animals treated in Schaudinn's fluid heated to 90° C. were nicely expanded with open peristomes and membranelles spread out.

Material, collected and prepared as described, was stained in a number of ways. The best staining was obtained by using Heidenhain's iron-alum haematoxylin very carefully differentiated in one-half per cent iron-alum. Both aqueous and alcoholic solutions of this stain were used. In general, the aqueous solution gave the more uniform results. It was used as described by Sharp (1914).

All material intended for permanent slides was treated by the centrifuge method. By this method, hydration, staining, differentiation, and dehydration may be rapidly and efficiently accomplished.

Material for sectioning was carried through as described by Sharp (1914). Sections were cut in series at five and ten microns. Sections were stained either on the slide or before imbedding.

Maximum optical equipment, which is essential to the best observations, was used throughout this investigation.

SYSTEMATIC POSITION

O. F. Müller (1786) recognized and described, under the name of *Trichoda inquilinus*, a marine tintinnid. Since that time numerous investigators, especially Brandt (1906, 1907), have revealed the extent and variety of these interesting forms. Kofoid (MS) regards the group as Tintinnoina, a subdivision of the heterotrichous ciliates.

GENERIC RELATIONS

The genus *Tintinnopsis* was founded by Stein (1867) for *Tintinnopsis beroidea.* The genus has been enlarged and emended by Daday (1887), Biedermann (1893), and especially Brandt (1907). More recently Jörgensen (1924) has further emended the genus *Tintin-*

nopsis. This author considers *Tintinnopsis nucula* and *T. ventricosa* as members of the newly proposed genus *Stenosemella* which he separates from other members of the old genus *Tintinnopsis*. This separation is made upon the firmer lorica and narrow, thin-walled collar about the rather constricted mouth in these two species. In his key to the genera he separates these two species from the remainder on the basis of their double lorical wall.

It seems best to retain the older and wider application of the name *Tintinnopsis* as defined until other evidences than these slight differences are available.

Systematic distinctions in this and in other genera of tintinnids are confined almost altogether to the structure of the lorica. In large measure this is justifiable since the lorica expresses in its structure the fundamental, peculiar constitution and behavior based on structure of the animal which resides within it.

SPECIFIC RELATIONS

Although a long series of observations have been made upon *Tintinnopsis* since the time of Fol (1884) it seems unwise to offer a complete list of synonyms under which *Tintinnopsis nucula* may have been known. An extensive series from widely separated regions is essential to a more complete understanding of the exact limitations of this form. For this reason and to avoid confusion, it seems best to always write *Tintinnopsis nucula* as attributed to (Fol) Laackmann. A list of the most significant synonyms includes:

Codonella nucula Fol (1884)
Tintinnopsis nucula Laackmann (1907), Brandt (1907), Wailes (1925)
Stenosemella nucula Jörgensen (1924).

Fol compares this species closely with *Codonella* (*Tintinnopsis*) *ventricosa* Fol, from which it may be distinguished by smaller size and by being less incrusted with extraneous matter. This species was first placed in the genus *Tintinnopsis* by Laackmann (1907).

Considerable variation in size is indicated by investigators of this species. The question of geographic races or local forms may enter into the final allocation of *Tintinnopsis nucula* and the related *T. ventricosa* (Fauré-Fremiet, 1924). These geographical races may integrate the two species completely. The variations due to the disturbing factors of growth and reproduction may enter into a consideration of these differences. At present, the greater size of *T. ventricosa* chiefly differentiates it from *T. nucula.* Extensive collection may either unite these two species or else indicate that *T. ventricosa* is rare; it may be absent from the California coast, although this is doubtful. For the present we must still consider these two species distinct.

The number of membranelles of the adoral zone in both *Tintinnopsis ventricosa* and *T. nucula* is twenty-two. This condition sets off *T. nucula* and *T. ventricosa* distinctly from other members of the genus *Tintinnopsis,* in which the number of membranelles is twenty or less.

HABITAT AND BIOLOGY

In common with most of the tintinnids, *Tintinnopsis nucula* is wholly marine in its distribution. Many species of tintinnids are found upon the high seas and some are found only under those conditions. Other species (particularly members of the genus *Tintinnopsis*) although fewer in number, are found near, or are limited to coastal waters. Among such neritic planktonts is *Tintinnopsis nucula.* This species has been recorded from nearly all seas. The writer has been able to obtain specimens at almost any time of the year in San Francisco Bay. Although no accurate quantitative counts have been made, it was noted that the animals were more abundant in the late spring months, especially after a great increase of diatoms or dinoflagellates. This condition was noted by Entz, Jr. (1909), for various tintinnids and has been accurately reported upon by Fish (1925) for *T. beroidea* in the restricted area of Woods Hole. In the bay waters few empty loricas were found, nearly every lorica containing live, normal animals. In the summer collections at La Jolla, however, where oceanic conditions prevail, there was a preponderance of empty shells. *T. nucula* is much less abundant at La Jolla than at San Francisco. It is a neritic species and is less delicate both in texture and viability than the genera and species of the high seas. Although *T. nucula* is a coastal form, in common with other members of the genus, transitions to brackish water have been reported (Steuer, 1910). This is easily understood if we consider the varied environment and salinity of harbor waters.

The writer was unable to cultivate or culture these animals in any way. They do not thrive in the laboratory under any conditions and frequently die within an hour of collection. A few will live in well aired, large culture dishes for about twenty-four hours at low temperatures.

Tintinnopsis nucula feeds principally upon diatoms, dinoflagellates, radiolarians, small chrysomonads, and bacteria. Sponge spicules are sometimes found within the cytoplasm. The remains of food organisms are commonly found within the cytosome in the posterior part, or their shells are found to be attached to the lorica as part of its structure. In numerous instances the silicious skeletons of radiolarians, spicules of sponges, and valves of diatoms are discharged by the anus and stored within the fundus of the lorica until the time of fission (pl. 15, fig. 38). Usually the smaller specimens of these organisms are used as food, although in some cases observed large diatoms and dinoflagellates were ingested (fig. B, *fd.b.*).

The type of food ingested indicates that *Tintinnopsis* is largely omnivorous in feeding habit. Both vegetable and animal foods are taken indifferently and both may be found in food vacuoles in the same animal (fig. B, *fd.b.*). After a diatom or dinoflagellate pulse these may predominate as the source of food.

GENERAL MORPHOLOGY

LORICA

The shell or *lorica* of *Tintinnopsis nucula* is elaborate. Brandt (1907) gives, in his monograph of the tintinnids, what is probably the best account of this complex lorica, but since he treats certain features incompletely I have given the description below.

The lorica of *Tintinnopsis* is usually yellow or brown in life. Part of this coloring matter is derived from incrusting, foreign bodies. The lorica is approximately olive-shaped, with the widest point within the upper third of the height and the truncate oral end (fig. A, *col.*). The lorica may also be described as vase-shaped, ovoid, beaker-shaped or cordiform, and it is thus described, particularly by Brandt (1907). It is closed aborally. Thus the lorica is rather short and wide. There is no caudal prolongation or pedicel as in *T. campanula* Daday (Fauré-Fremiet, 1924, fig. 29). The lorica is of firm consistency. It usually tears when sectioned. The body (fig. A, *bod.*) of the lorica gives no evidence of any distinct helicoidal coiling such as is prominent in the collars of the genus *Codonella* or in the body of *Coxleilla annulata* (Entz, Jr., 1909, pl. 10, fig. 1).

The *nuchal band*, surrounding the base of the collar, is always distinct (fig. A, *nu.b.*). It separates the collar from the body of the lorica and is a thickened band belonging properly to the body of the shell rather than to the collar or its base. The lorica is about two microns in thickness throughout, without the foreign, irregular bodies. It may be slightly thicker near the *fundus* (fig. A, *fun.*).

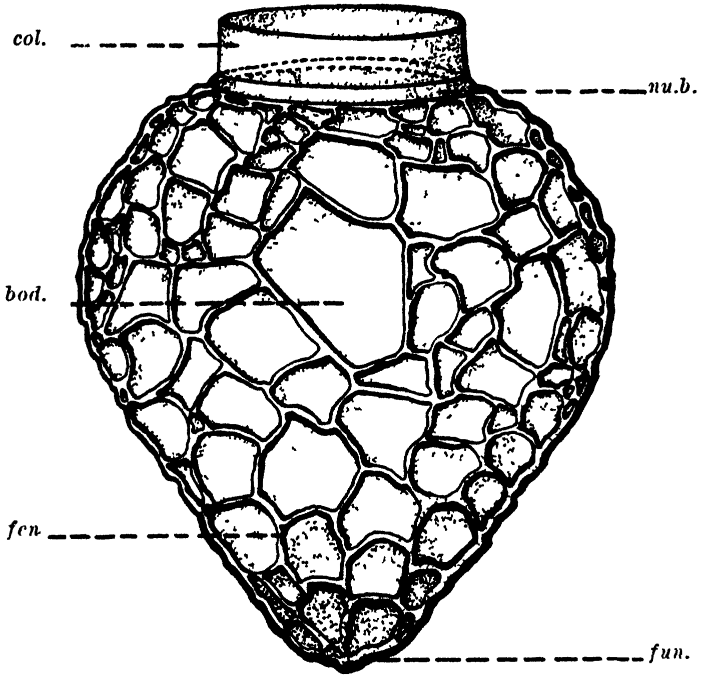

Fig. A. Semidiagrammatic figure of the lorica of *Tintinnopsis*. Abbreviations: *bod.*, body; *col.*, collar; *fen.*, fenestrations; *fun.*, fundus; *nu b.*, nuchal band. × 1150.

The *collar* (fig. A, *col.*) surrounds the oral aperture of the lorica and arises inside the nuchal band. It may consist of one, or at the most two, non-separable annuli. The disposition of these rings is probably correlated with the slight spiraling of the somatic ciliation and of the ciliary membrane (pl. 12, figs. 6, 8) which aids in the shaping up of the lorical components. The collar is frequently broken off, possibly during life. It is usually without reticulations or other structures and seldom has a great mass of agglutinated foreign bodies upon it (pl. 12, figs. 6, 8). Windows, characteristic of *Dictyocysta*, are never found. However, in certain loricas found in the open sea there is a marked tendency toward the formation of irregular, halfmoon-shaped openings in the collar. The collar is less firm in consistency than the remainder of the shell. The margins of the collar are smooth and without teeth or other ornamentation except as this appearance is given by added foreign matter. Both the collar and the body of the lorica are possibly colloidal (Entz, Jr., 1909).

There is no indication of a lorical diaphragm such as that found regularly in *Codonella* and similar forms. Entz, Jr. (1909, pl. 8, fig. 16), figures a lid-like detritus mass closing the oral aperture in *Codonella orthoceras* Haeck. This massing of irregular materials I have found in *Tintinnopsis*. It represents an aggregation drawn into the aperture by the membranelles and may be protective in function or concerned in closing off the lorica preparatory to encystment.

The lorica has a reticulate structure (fig. A, *fen.*). The wall is thin, translucent, and apparently structureless between the elevated margins of the external reticulations. The inside of the shell is smooth and with but very slight indications of this external fenestration. The elevated ridges stain deeply with iron-haematoxylin.

Coating the exterior of the lorica there is a varying amount of agglutinated matter. Many of these particles are built into the intimate structure of the lorica. For the greater part this detritus is faecal, consisting of bits of radiolarians, diatoms, sponge spicules, or other bodies of organic origin; although other highly refractive bodies are met with which may be formed from the substance of the shell. This extraneous matter often conceals the real structure of the lorica (Calkins, 1902). The cavity of the lorica frequently contains a mass of stored faeces and secreted foamy matter (pl. 15, figs. 31, 38), which is used by the freed daughter animal at the time of binary fission to construct a new lorica. There is no groove or other special structural adaptation for the reception of this mass in *Tintinnopsis* (pl. 15, figs. 31, 38).

The size of the lorica is variable but within certain definite limits. In a measured series of loricas the average external dimensions were forty-two microns in length, from the extreme aboral apex or fundus (fig. A, *fun.*), to a line drawn at the center of the diameter of the oral aperture at the base of the nuchal band. At the oral end the collar, above the nuchal band, averages nearly six microns in height. The widest point of the body of the lorica is at a point about two-thirds above the fundus. At this position the lorica may measure about thirty microns across. The length-breadth relation is thus about 1:1.5. In diameter the collar averages twenty microns at the base.

The diameter of the aperture of the collar is slightly greater than at the base. There is considerably less variation in these measurements than those reported by others (Brandt, 1907; Entz, Jr., 1909; Jörgensen, 1924).

CYTOSOME

Although there is an extensive literature dealing with the general morphology of marine ciliates, little actual cytological work has been attempted. Studies upon the cytology of *Tintinnopsis* of various species have been made by Entz, Jr. (1909). Other papers of general nature include those of Schweyer (1909), Merkle (1909), and Fauré-Fremiet (1908, 1924). These papers deal with numerous genera and species of tintinnids.

GENERAL ACCOUNT

The highly contractile animal of *Tintinnopsis nucula* measures when fully expanded nearly seventy microns in length from the stalk at the base to the base of the reflexed peristome margin. The diameter of the cytosome is partly conditioned by its state of expansion. In a fully expanded animal it is slightly less than the circumference of the collar, or about twenty microns in diameter. Careful comparisons show these measurements to be closely corresponding to those of the living animal, the reagents used causing but slight shrinkage. In a fully extended swimming animal about one-half of the organism may be projecting freely above the mouth of the collar. A disturbed animal may, in *T. nucula,* completely withdraw into the lorica and fill the larger part of its cavity.

Tintinnopsis nucula is colorless and refractive. Within the cytoplasm, material derived from ingested diatoms and flagellates forms yellow vacuoles.

According to Fauré-Fremiet (1924, fig. 31), the body of some species of *Tintinnopsis* is attached to the bottom of the lorica without a distinct pedicel. *T. ventricosa* is figured with a terminal attachment by this author. The attachment of *T. nucula* is certainly not similar. Entz, Jr. (1909), failed to find any indication of the attachment but presumed it to be terminal. The attachment of *T. nucula,* as also in *T. ventricosa,* is without a distinctly differentiated pedicel and may best be described as characteristically subterminal (pl. 12, figs. 3, 5) within the fundus of the lorica, as seen in both living and fixed animals. The region of attachment is always below the cytopyge and it terminates the column (fig. B, *at.*). The attachment is derivable from a pseudopodial type characteristic in the Stentoridae and described by Fauré-Fremiet (1908). It is somewhat comparable to that described by Schweyer (1909) in *Cyttarocylis ehrenbergii* or to that in *T. lusus-undae* which I have repeatedly watched in the living plankton at La Jolla.

The *column* (fig. B, *clm.*) in the extended state is narrowly campanulate or pyriform, approaching gradually apically to the point of attachment (fig. B, *at.*), or sometimes rather abruptly (fig. C, *at.*), and increasing in diameter orally. In transverse section it is circular or subcircular. At the oral or distal end it broadens to a spreading, distinct, expanded, thickened peristome collar (fig. C). The column is cylindrical and its ventral and dorsal aspects may be distinguished by the position of the cytopyge (fig. B, *cytop.*) and by the continuation of the broad ciliary membrane and lateral field (fig. B, *cil.m.*) slightly spiraling down part of its length, both on the ventral surface (pl. 12, fig. 5). The columnar surface is covered by a thin, unornamented pellicle. Body cilia follow the twenty-two longitudinal myonemes, the alternating spaces between them being hyaline bands. At a point slightly below the peristome collar the ventrosinistral side of the column is expanded into a *lateral lobe* (fig. B, *lobe*) which is highly plastic and capable of being thrust in and out. This lobe is frequently siderophile. In certain other members of the genus *Tintinnopsis* it is a fixed, rigid structure. It was thus described by Fauré-Fremiet (1924) in *Tintinnopsis campanula* and I have been able to determine its permanance in *T. dadayi.* The functions and activities of this lobe I will describe in a later section. The column is unsupported by a cytoskeleton such as that found in the genus *Strombidium* (Anigstein, 1913). It depends upon the turgidity of the cytoplasm for support.

The *peristome collar* (fig. G, *cl.*), which is the thickened edge of the flaring and truncated oral end of the column of the animal, always lies nearly in the same plane, that is, its distal end is not noticeably displaced anteriorly or posteriorly. It is located peripherally to the membranelle zone. When seen face on it appears as a single, flat spiral enveloping the peristome field. The spiral condition obtains also in *Stentor* and in other Heterotricha (Johnson, 1893), but is generally steeper. The direction of the spiral is left-handed, or leiotropic, viewed from below. Between the distal and proximal ends of this spiral is a narrow, deep space or *cleft* (fig. E, *cleft*) and through it the ciliary membrane passes vertically down the surface of the column, on the inner side (figs. B, E, *cil.m.*) of the peristome collar. This ciliary membrane, which is a neuromotor structure and a direct outgrowth and transformation of the distal end of the adoral membranelle zone, arises from it in a manner to be described later.

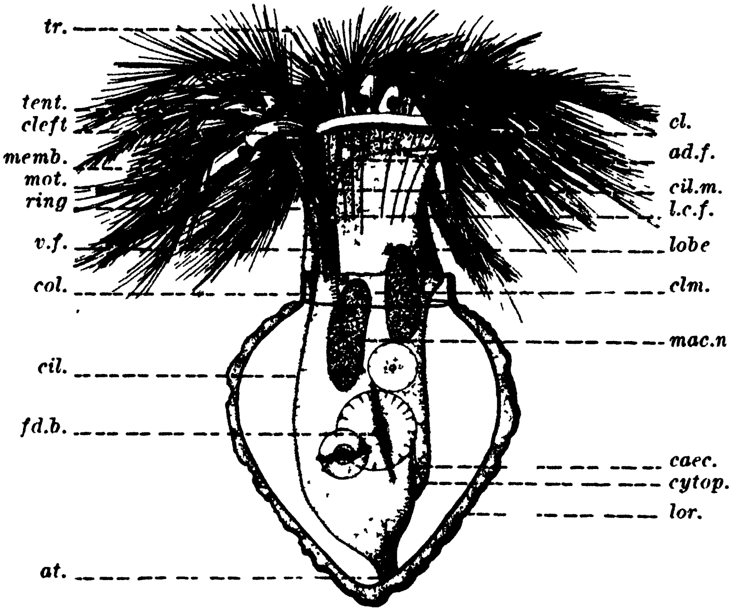

Fig. B. Semidiagrammatic figure of *Tintinnopsis* showing the general morphology of the cytosome. Abbreviations: *ad.f.*, adoral fiber of the neuromotor apparatus; *at.*, attachment to the lorica; *caec.*, caecum; *cil.*, somatic cilia; *cil.m.*, ciliary membrane; *cl.*, peristomal collar; *clm.*, column; *cleft*, cleft in the peristomal margin; *col.*, lorical collar; *cytop.*, cytopyge; *fd.b.*, food body; *lobe*, lateral column lobe used in shell-building; *lor.*, outlines of the lorica; *l.c.f.*, lateral ciliary field; *mac.n.*, macronucleus; *memb.*, membranelle; *mot.*, neuromotorium; *ring*, circumoesophageal ring; *tent.*, tentaculoid; *tr.*, trichocysts; *v.f.*, ventral fiber of the neuromotor apparatus. × 850.

The plane of the peristome collar is either somewhat oblique (fig. G) or nearly perpendicular to the main axis (fig. B) of the column. The oral surface of the collar, which may be about five microns wide, carries on its oral face the crown of membranelles and tentaculoids (fig. E, *memb.*, *tent.*). The proximal end of the whorl ends somewhat abruptly and the point where it ends carries three membranelles which enter the infundibulum. The distal end tapers as it approaches the cleft (fig. E, *cleft*) and overlaps the proximal end for a short distance.

The *peristome field* (this term, as used in this paper, denotes the same series of structures described by the term frontal field in *Stentor* and related forms, and is more nearly descriptive of these organs in *Tintinnopsis*) is concave and somewhat disc-shaped (figs. D, E). It occupies nearly the whole of the anterior or oral end of the animal except the peristome collar, the inner margin of which limits it, and together with its own structure and associated structures forms one of the most complex parts of the whole organism. It is a deep, adorally spiraling plate which arises from the inner margin of the collar, as described, and passes downward, or adorally, into the excentrically placed *infundibulum* (fig. G, *inf.*). Its surface is marked by striations, regularly placed with bands of clear substance between, spirally disposed, and entering the infundibulum. Dorsolateral of the infundibulum and within the peristome field there arises a highly mobile, prominent eminence or *oral plug* (fig. D, *o.p.*). The whole peristome field is covered with a thin pellicle which like the collar surface is never ciliated.

The structure which I have designated as the *oral plug* (fig. D, *o.p.*) is a most remarkable organ. Its physiology is described in a following section on the living animal. The oral plug forms one of the most characteristic organs of the tintinnids. On account of its extreme mobility and prominence, it was early noted and described by Stein (1867) and called by him the piston-organelle. This term is probably not descriptive of its function in the living animal, hence I have redesignated it. This oral plug may be homologous with the less conspicuous structure of the same name in *Balantidium* (McDonald, 1922), or in *Diplodinium* (Sharp, 1914). Its great mobility, situation, and sensitivity may be taken as evidences of this homology. Very delicate fibers may run between the surface markings of the peristome field (Entz, Jr., 1909). These are probably neuromotor fibers.

The organs of feeding may be considered as made up of the infundibulum, cytostome, and gullet. The pre-oral cavity or *infundibulum* (fig. D, *inf.*) descends from the peristome field, plunging into the slightly spiral gullet. No marked structural changes are associated with it. It forms a deep funnel-shaped depression in the peristome field which narrows as it progresses. It may be as much as three-eighths the breadth of the peristome field. Its wall is lined by the regularly placed membranelles and associated accessory combs. The mouth or *cytostome* is the entrance to the gullet. It is marked by no special structural modifications and through it food pellets are passed into the gullet below.

The *gullet* (fig. D, *gul.*) leads directly from the mouth. It forms a curved tube, the convex side of which is directed toward the outer body wall and the concave side toward the oral plug. It is frequently as much as one-quarter or even one-half the length of the column. Its diameter at a point near the mouth may be about ten microns when fully extended. In section it is circular. There are no special internal myonemes, retractile strands, or other contractile elements surrounding or associated with these organs of food-taking, as in *Diplodinium* (Sharp, 1914). The gullet is covered by a thickened pellicle and extending down its length are several oral membranelles. These are made up of short cilia that arise from the adoral crown and course down the gullet in a lengthened spiral. Accompanying them are accessory combs. The infundibulum and gullet are very extensible and allow the passage of large objects such as whole diatoms more than twice the diameter of these organs when contracted.

A prominent *cytopyge* (fig. B, *cytop.*) marks the lower part of the column of the animal on the ventral surface at a point slightly above the attachment to the lorica. The cytopyge is a papilla or raised, clear, elongated point in the pellicle. When fully open it may extend inward as a tube and may be distinguished as being divided into two parts, a *caecum* (fig. B, *caec.*) and a *cytopyge* (fig. B, *cytop.*). The caecum is a tubular structure while the cytopyge is slot-like. The caecum is anterior to the cytopyge and extends upward and behind it. There are no supporting structures in connection with these organs as are seen in the Ophryoscolecidae (Sharp, 1914).

These organs of defecation serve the organism in the function of the discharge of the solid remains of food. Through the cytopyge the indigestible valves of diatoms and the plates composing the cuirass of dinoflagellates are passed, as well as other faecal material. Fluid excretory products are probably discharged through the cytopyge in part, and in part by the general surface of the body, there being no contractile vacuole. Although I have examined many animals, both living and preserved, I have at no time been able to distinguish any evidence of a contractile vacuole. Entz, Jr. (1909), lists a series of both marine and fresh-water forms for which he claims at least one of these vacuoles, opening generally into the infundibulum. In one species, *Tintinnidium pusillum,* he reports a non-contractile vacuole. In no case have I been able to confirm either this observer or others

(Entz, Sr., 1884) regarding these asserted vacuoles. It is entirely possible that they may be simply food vacuoles which may be readily found in this region of the body.

ECTOPLASMIC STRUCTURES

The whole surface of the body is coated with a thin, delicate, scarcely distinguishable *pellicle* which is slightly less than one micron in thickness. That part which covers the peristome surface and the peristome collar and related structures which extend into the gullet, is somewhat thicker and stains intensively with iron-haematoxylin. The pellicle about the cytopyge is also somewhat thicker. I have been unable to distinguish in the pellicle any evidences of striations or other markings. The pellicle is highly flexible, allowing the very mobile cytoplasm within to throw it freely into folds. It is retentive and protective as well as supporting in function.

A highly differentiated and separated *ectoplasm* is not clearly evident in *Tintinnopsis nucula.* There is no boundary layer (Sharp, 1914; McDonald, 1922) or other definite, distinguishable line of demarcation between the ectoplasm and endoplasm in this form. The large alveoles of the endoplasm grade into the smaller, delicate ones of the periphery which compose the ectoplasm. Deeply staining granular bodies of the endoplasm may extend well toward the ectoplasm. At the extreme periphery the protoplasm becomes more dense and very finely granular.

Extending inward, and equidistantly placed in transection, are narrow (less than two microns across) hyaline bands or "*Zwischenstreifen*" which run vertically. Beside these clear areas the cilia rise from basal granules which are somewhat deeply set into the outermost region of the granular portion of the ectoplasm. This structural differentiation of the ectoplasm into clear areas and finely granular *myonemes* (fig. C, *my.*) recalls the structure of the parasitic *Balantidium* (McDonald, 1922). So far as I have been able to determine, in both living and stained organisms these granular and hyaline bands do not branch. My interpretation of these structures confirms closely that of McDonald (1922) and need not be further dwelt upon here. It corresponds closely to that of others (Maier, 1903) and is probably general in the Heterotricha.

The myonemes fade posteriorly and are difficult to follow. They usually meet at the stalk. Anteriorly, they extend into the reflexed margin of the collar and possibly connect with the basal granules of the adoral membranelles. There is a little evidence that these granular myonemes are made up of alternating clear and denser matter, although the small size of *Tintinnopsis* makes it difficult to determine this fact satisfactorily. The myonemes constitute one of the most powerful effector systems in the organism.

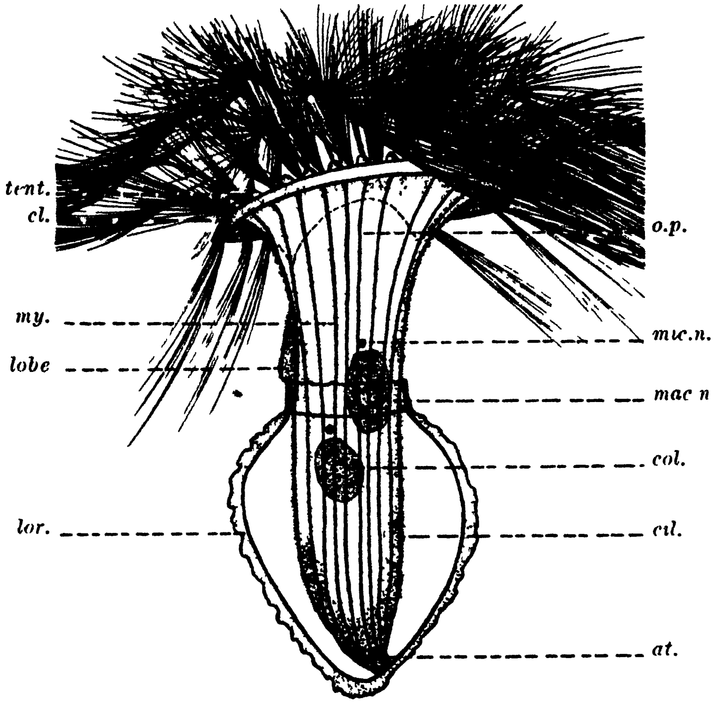

Fig. C. Semidiagrammatic figure of *Tintinnopsis* showing especially the myonemes and other external features. Abbreviations: *at.*, attachment to the lorica; *cil.*, somatic cilia; *cl.*, peristome collar; *col.*, lorical collar; *lobe*, lateral column lobe. *lor.*, lorica; *mac.n.*, macronucleus; *mic.n.*, micronucleus; *my.*, myonemes; *o.p.*, oral plug; *tent.*, tentaculoid. X 850.

A peculiar system of intensively staining, elongate *trichocysts* covers the collar and tentaculoids and follows the protoplasmic accessory combs into the peristome in many tintinnids (figs. D, E, *tr.*). Some of these bodies may even enter into the wall of the infundibulum. Entz, Jr. (1909), by whom they were especially noted, homologizes them with the trichocysts covering the surface of many ciliates. This author states that Stein (1867) may have had them in mind, in describing "*Trichocysten*" in the fresh-water tintinnid, *T. fluviatile*.

In *Tintinnopsis nucula* these trichocysts are clustered especially about the tentaculoids (fig. B, D, *tr.*). A few were found scattered in a random manner in the peristome or upon the collar. In size they vary from one and one-half to two microns long by about one-half micron in breadth. No indication of their function has been determined by a study of the living animal. The presumption that they represent trichocysts is a very strong one although there is no indication of trichocyst structure. Their clustering about the tentaculoids may indicate that these organs are concerned with the capture and paralysis of small flagellates and other organisms used as food, or that they have a protective action as in *Paramecium* (Jennings, 1906). No indication of expelled threads have been noted in my preparations. No regularity of arrangement can be determined for them. There is no evidence that these structures are taste organs or tactile organs. If such were the case it might be expected that there would be demonstrable connections with the neuromotor system. Such is not the case. In *Paramecium,* the trichocysts are connected with, and are part of, the neuromotor system (Rees, 1922). In *Tintinnopsis,* on the other hand, there is no clear evidence that this is the case.

Fauré-Fremiet (1924) describes, in *Tintinnopsis ventricosa* and in *T. campanula,* a single series of stiff, elongate cirri surrounding the collar and extending from the column. These cirri are asserted to act as supporting rodlets which aid in keeping the animal extended when swimming. I have been unable to find any evidence of these cirri in either the living animal or in permanent preparations. They are certainly absent in *T. nucula* and it is suggested that they may constitute a specific distinction between these closely related forms. I have been unable to find them in *T. campanula* among any of my own preparations.

The *tentaculoids* make up a distinct ectoplasmic modification peculiar to the tintinnids. They were early noted by Haeckel (1873). They are especially prominent in the genus *Tintinnopsis* (Fauré-Fremiet, 1924; Jörgensen, 1924). Athough I feel that these organs, from their position, shape, association with trichocysts, and connections with the gullet may be neuromotor structures, demonstrable connections with the neuromotor system have not been shown by any of my preparations. The small size of these organisms has so far prevented the determination of these exceedingly fine elements. It is highly probable that they may be connected with a peripheral sensory network not unlike that of the lattice structure in *Euplotes* (Yocum, 1918).

The *tentaculoids* (fig. B, *tent.*), when expanded, are ball-shaped or claviform structures which rest upon a short pedicel and are arranged between the adoral membranelles upon the peristome collar. There is one of these tentaculoids beside each membranelle (fig. C, *tent.*); they are thus the same in number as the membranelles. If examined in the living animal, it may easily be seen that the tentaculoids are retractile and may be freely withdrawn almost completely into the substance of the accessory combs themselves, so that they are lost to view. This observation I have confirmed upon fixed and stained animals (figs. B, C, D, E, *tent.*) in which the tentaculoids are found in all degrees of expansion and retraction.

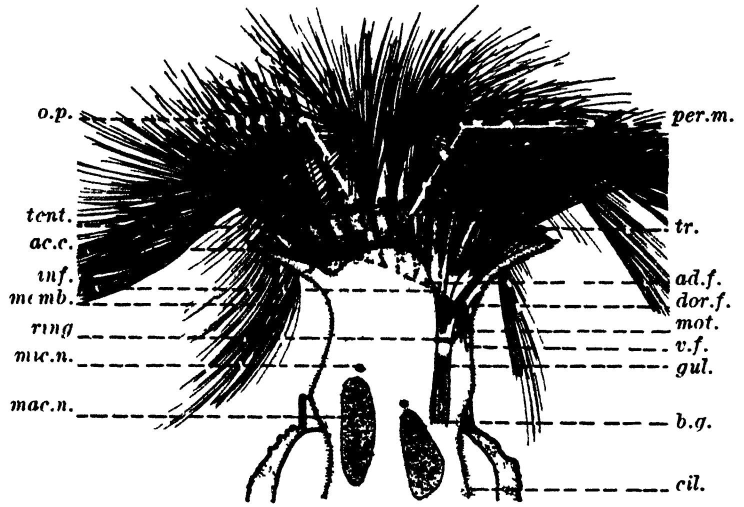

Fig. D. Semidiagrammatic figure of median sagittal section through the anterior portion of *Tintinnopsis*. Abbreviations: *ac.c.*, accessory comb. *ad.f.*, adoral fiber; *b.g.*, basal granules; *cil.*, somatic cilia; *dor.f.*, dorsal fiber; *gul.*, gullet; *inf.*, infundibulum; *mac.n.*, macronucleus; *memb.*, membranelle; *mic.n.*, micronucleus; *mot.*, neuromotorium; *o.p.*, oral plug; *per.m.*, peristome margin; *ring*, circumoesophageal ring; *tent.*, tentaculoid; *tr.*, trichocysts; *v.f.*, ventral fiber. × 850.

Extending from the proximal end or base of each tentaculoid is an *accessory comb* (fig. E, *ac.c.*). These accessory combs are narrow and conical, being widest at their distal part, and they extend to the floor of the peristome field in a slightly spiral course following the membranelles into the infundibulum. The accessory combs are hyaline, ectoplasmic structures and seem to bear no direct relation to the neuromotor system (figs. D, E, *ac.c.*). Trichocysts may be scattered over the surface. Under certain conditions the tentaculoids stain intensely with iron-haematoxylin and the accessory combs are also equally stained (pl. 12, figs. 5, 9); both structures then appear homogeneous and dense. At other times they seem to take less stain and are clear and without structure (pl. 12, figs. 1, 7). The tentaculoids and accessory combs in the peristome field constitute the "*Begleitkämme*" and "*Deckplättchen*" described by Entz, Jr. (1909), or "intercalary cirri of the adoral ciliary plates" (Kofoid, 1905).

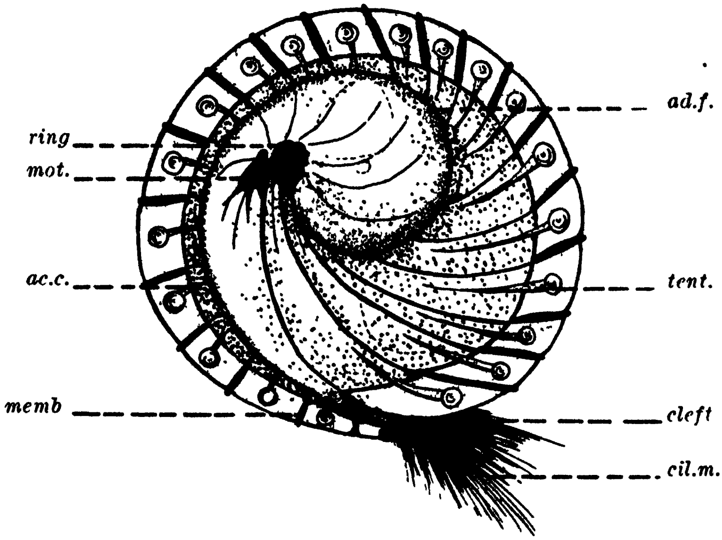

Fig. E. Semidiagrammatic figure of *Tintinnopsis* from above downward showing the peristome field. Abbreviations: *ac.c.*, accessory comb; *ad.f.*, adoral fiber; *cil.m.*, ciliary membrane; *cleft*, cleft in peristome margin; *memb.*, membranelle; *mot.*, motorium; *ring*, circumoesophageal ring; *tent.*, tentaculoid. × 2150.

ENDOPLASMIC STRUCTURES

The *endoplasm* includes the great trophic mass of the cytosome together with its inclusions. It is surrounded by the thin and slightly differentiated ectoplasm. The endoplasm is highly fluid, especially during the active periods of feeding and division. The mobile oral plug is largely made up of endoplasm. Posteriorly the stalk may change rapidly from a stiff gel to a fluid sol and become detached and then reattached in a short time.

I have observed few clear indications of flowing currents within the endoplasm. Tracing the food vacuoles in fixed specimens has enabled me to determine that the course of these vacuoles is almost directly anterior-posterior in direction. Often they remain in the aboral part of the organism, which serves as a region for the storage of undigested food (fig. B, *fd.b.*). No one who has watched the living animal can fail to be impressed with the great mobility of the organism and the fluidity of its contents.

The endoplasm of fixed and stained specimens is highly alveolar; more rarely is it granular. The collar and peristomal regions are made up of a denser, less alveolar cytoplasm while the column is alveolar to a greater extent. The living animal appears to possess clear and very finely alveolar protoplasm.

That the *mitochondria* or chondriosomes are cell organs is a well established fact (Cowdry, 1924). In *Tintinnidium inquilinum,* Fauré-Fremiet (1908) established the presence of phospholipin spherules about one micron in diameter which he claims are organized into a mitochondrial apparatus visible in the living animal. In living specimens of *Tintinnopsis nucula* treated by a special technique (Causey, 1925), I have been able to demonstrate rodlets staining deeply with Janus green B. No regularity of arrangement or number can be made out, nor are there any evidences of division at the time of binary fission. These results agree with those of Causey (1925) upon *Paramecium caudatum.* From their relationship to food vacuoles it seems clear that these rodlets are connected with metabolism. Spherical mitochondria associated with katabolism are rare or absent.

Vacuoles constitute a prominent feature of the endoplasm. These are largely filled with the remnants of diatoms, dinoflagellates, or other articles of diet, and some of which lend their coloring matter to *Tintinnopsis.* These food vacuoles occupy, mainly, the posterior parts of the organism. Here they may await complete digestion and persist for a long time.

Within the endoplasm of some individuals (especially during the period of fission) may be found a loose massing or cloud of *chromidial substance* (Cowdry, 1924) near the region of the origin of the newly forming peristome. These chromidial granules stain deeply with iron-haematoxylin, borax-carmine, or with eosin. The question of their origin and fate will be dealt with in a later section.

NUCLEI

The nuclear system of *Tintinnopsis nucula* is made up of two macronuclei and two micronuclei, constant in form and size in the normal interkinetic condition. They form the most striking landmarks in a stained preparation, lying slightly below the middle of the upper half of the organism (fig. C, *mac.n.*). In the living animal they are scarcely visible but may be brought out with dyes, especially aceto-carmine or methyl-green. It is evident from fixed and stained material (especially at the time of mitosis and binary fission) that the nuclei, due to active changes in the endoplasm, may shift their position within the animal. They usually retain their position in the middle region at a point near the outer wall of the column near the ventral side (pl. 12, fig. 5). This condition is especially notable at the time of binary fission (pl. 14, fig. 16). Each micronucleus is associated with one macronucleus.

The two *macronuclei* lie separated and equidistant from the right and left sides of the animal, one somewhat below the other (fig. B). They are entirely distinct and without any indications of commissures or connections of any kind. In no wise can they be said to be disassociated segments of a single nucleus nor are they comparable to the nuclear condition of *Stylonychia* (Lang, 1901). They form two distinct somatic, cell nuclei.

In form and size the two macronuclei are alike. They are usually bean- or kidney-shaped (pl. 12, fig. 9) or more rarely assume an ovoid, round, or even irregular form (pl. 12, fig. 3), possibly due to disturbing features in reorganization. In these latter cases there is frequently a vacuole surrounding the nucleus with its wall at some distance. Each nucleus is rarely more than twenty microns in length and about eight microns in diameter, with slight variations in proportion.

The *finer structure* of the macronuclei does not seem to have been particularly determined in *Tintinnopsis nucula.* Entz, Sr. (1884), determined that in a dying tintinnid the macronucleus, upon disintegration, discharged granules of various sizes. Entz, Jr. (1909, pl. 20, fig. 5), figures a macronucleus containing granules differing in size, some with vacuoles around them. To these vacuoles I shall refer later in my consideration of nuclear division and parasites.

Each macronucleus has a distinctly staining, rather heavy nuclear wall (pl. 15, fig. 22) toward the ends of which the chromatin granules which compose the mass of the nucleus are less evident (fig. C, *mac.n.*). In optical section this area is clear. In a favorably stained nucleus the chromatin substance is found to be aggregated into dense, black masses or granules of different sizes. Some of these are about one or two microns, or even three microns, in their greatest diameter. Other granules are scattered between these greater masses which stain intensely with borax-carmine. No regularity of arrangement of the larger granules through the nucleus can be made out. Usually there are about twenty of these larger masses, although the number may be less. I have been unable to determine the cytological significance of these granules. They may represent the so-called macrosomes and microsomes (Anigstein, 1913; Bishop, 1923). As in *Balantidium* the larger granules may be trophic chromosomes (Hegner and Holmes, 1923). In a deeply stained nucleus the smaller masses as well as the larger are stained equally with iron-haematoxylin. The artificial division of chromatin granules into the so-called macrosomes and microsomes is probably a matter of physical chemistry and not of a fundamental division of chromatin into two contrasting nuclear substances. Wilson (1925) has used the term microsome in an entirely different sense to refer to the smaller cytoplasmic granules, hence the term should not be applied to nuclear granules. The macronucleus stains a uniform deep orange with Mallory's connective tissue stain.

The two *micronuclei* (fig. F, *mic.n.*) are alike in form and structure. A description of one will fit the other. Each is exceedingly small. In the resting condition or interkinetic period, the micronucleus may be about three microns in diameter. It is ovoid or globular. It may rest in a dorsolateral position, toward the upper or oral end of the animal, in a depression in the macronucleus. More often the micronucleus is separated at some distance from the macronucleus, but it may come to lie at almost any position within the organism not more than ten microns away from the macronucleus. The interkinetic micronucleus is spherical or subspherical with the flattened side at right angles to the longitudinal axis of the macronucleus. It is surrounded by a nuclear wall. Within the micronucleus there are numerous small granules. Usually the micronucleus is only slightly stained so that no structure can be determined within it. Often it appears as a single large homogeneous mass (fig. C, *mic.n.*). Each micronucleus always retains its identity and generally maintains itself in rather intimate association with one of the macronuclei.

NEUROMOTOR APPARATUS

The term neuromotor apparatus denotes a system of integrated fibers associated with the motor organs and connected with a central, massive body or motorium. Because this system of fibers connects with the motor organs and is integrated to the motorium, it is credited with the function of coordinated conductivity of neuroid excito-motor impulses (Sharp, 1914; Kofoid, 1916; Taylor, 1920; Herrick, 1924).

Since the neuromotor system of *Tintinnopsis nucula* is intimately associated with feeding, locomotor, and other reactions of the organism, I will treat in this section not only the fibers and motorium but will also include the ciliary membrane, somatic cilia, and membranelles as part of that system. The myonemes, tentaculoids, and trichocysts have already been dealt with above, and while they constitute motor or effector organs, the lack of clearly determined fibrillar connections of these organs and the others treated in this section has led me to consider them separately. It must be understood that by so doing I do not consider the myonemes, tentaculoids, and trichocysts necessarily independent effectors. A more refined technique used upon these small ciliates may reveal evidences of intimate connections between all these organ systems.

To Entz, Jr. (1909), credit must be given for having recognized and described fibrillar elements in the collar which descend into the region of the gullet. He failed to comprehend their significance and did not connect them in any way with a motorium. He concluded that the motor functions of contraction reside in the myonemes (myophanes), or within the plasma itself.

The *neuromotor system* of *Tintinnopsis* is composed of seven distinct parts. These are (1) the membranelles, (2) the ciliary membrane, (3) oral membranelles, (4) the somatic ciliation, (5) the fibrillar connecting system, (6) the circumoesophageal ring, and (7) the central neuromotorium. These parts are intimately connected in a unified system. I shall treat them in order.

The *adoral membranelles* are twenty-two in number. No differences in number have been observed. Each is about twenty-five microns in length, measuring from the extreme distal tip to the margin of the thickened collar. They form a leiotropic, spiral crown about the collar, with which they are connected, and pass into the peristome where at the base they are united to the adoral fiber from the motorium. Beside each membranelle is a tentaculoid. Three of the adoral membranelles descend into the infundibulum where they are gradually modified in structure to become the oral membranelles and plunge into the gullet.

The adoral membranelles are blade-like (fig. B, *memb.*). They compare closely with the membranelles of *Diplodinium*, but are flat, blade-like ciliary brushes. The outermost cilia of each membranelle are especially thickened and rather rigid, forming primary cilia. These cilia afford a rigid support against which the remainder of the components may beat since they are held out rather stiffly at right angles from the body. Their distal ends are held less rigidly and beat more freely. At the base of these membranelles are three basal granules which are about two microns in diameter. They are connected by fibrils in such a manner that each one forms the apex of an equilateral triangle with the base uppermost. Through the base passes the adoral motor fiber.

At the base of the flat, blade-like membranelles the innermost cilia are frequently distinctly separated, forming a row which has been taken to be a distinct ciliary line (Entz, Sr., 1884). In prepared specimens or in dying animals, this appearance is common. If a fresh specimen is examined, it is found that this row is actually only a part of the adoral series of the innermost row of the cilia which make up the adoral membranelle. This may be confirmed upon prepared material (pl. 12, fig. 9).

The cilia which compose the adoral membranelles are somewhat loosely held together by a thin, delicate coating which is dissolved away by reagents or at the disintegration of the living animal, thus allowing the component cilia to become separated.

The adoral membranelles do not seem to possess the long ciliary rootlets described by Sharp (1914) in *Diplodinium.*

The *ciliary membrane* descends on the ventral surface of the column (fig. B, *cil.m.*) from the distal end of the adoral zone. This membrane was first noted by Fauré-Fremiet (1924) and described by him as a *"frange bordante."* The author failed to find any connection between this membrane and the adoral membranelles.

Before proceeding with the description of this organ, I will call attention to the fact that this membrane is not a true undulating membrane (Minchin, 1922). The term undulating membrane is best reserved for the structure found in flagellates only, particularly the trypanosomes and intestinal Polymastigina. In the flagellates of these types, the undulating membrane is a projecting, folded structure built of pellicle or cuticle which carries with it an internal marginal fiber connecting with the blepharoplast, and usually with a parabasal body. In the ciliates, on the other hand, the organs described as undulating membranes are never of this structure (Chambers and Dawson, 1924); instead they are made up of fused rows of cilia, each cilium arising from a basal granule. It seems best to differentiate these analogous structures by a term which will not convey the idea of homology. Hence I propose the name "ciliary membrane" to describe, and at the same time convey the idea of possible functional identity for, these structures in ciliates.

The ciliary membrane of *Tintinnopsis* is a structure which finds no parallel in other ciliates. It is a system of elongate, highly flexible cilia, arising from rather large, globular, basal bodies (fig. G, *cil.m.*). The cilia which arise from these basal granules extend in a single line down the ventral surface of the organism rather more than half the length of a fully extended animal (pl. 12, fig. 5). They form a continuation of the adoral membranelle system and arise by a metamorphosis of the cilia composing that series. There is a gradual change in their form, as they pass over the peristome collar cleft, comparable to the changes in the adoral and oral membranes in *Diplodinium* (Sharp, 1914) but opposite in its direction. The longest cilia which compose the ciliary membrane are those nearest the adoral membranelle series from which they can be distinguished because they are single cilia only. They are about the same length as the adoral series at their adoral origin. As the membrane proceeds down the length of the body the cilia grow progressively shorter until they reach a point where they are indistinguishable from the body cilia in length.

This ciliary membrane functions in house-building and repair. It is the *house-builder* and aids in the removal of faecal wastes by wafting detritus outward and away from the lorica. The possibility that the ciliary membrane may store up detritus from the outside by bringing materials into the lorica is expressed in another section. The powerful current set up by the membrane sweeps these masses from their storage place within the lorica and carries them forward until they are out of the collar. At this point they are picked up, partly by the membrane and partly by the active adoral membranelles, and plastered over the lorica (pl. 15, figs. 31, 38). This ciliary membrane is connected through its basal granules by fine fibrils to the adoral fiber from the motorium. *The importance of this matter is that lorica-building,* which was suggested especially by Kofoid (1905) to take place through the *activities* of the animal, *is actually a function of the neuromotor system, acting particularly through the ciliary membrane and membranelles.* The *lorica* is then, in part at least, *a construction;* it is a result of coordinated activities, dependent for its being upon the resultant activities of a central, dominating neuromotor system (pl. 15, figs. 31, 38). I shall return to this matter later.

The *oral membranelles* (fig. D, *memb.*) are three in number. They arise by a sudden differentiation and change from the blade-like adoral membranelles to flat plates composed of fine cilia which continue their course in a slight spiral down the length of the gullet. I can add very little to the excellent descriptions of these organs given by Johnson (1893) in *Stentor,* and McDonald (1922) in *Balantidium,* with which they compare closely. Each membranelle ends at the base in the gullet in a distinct globular basal body (fig. D, *b.g.*).

The *somatic ciliation* of *Tintinnopsis* is confined to the column. The cilia which make up this series are short, less than two microns in length, and are very difficult to see in the living animal. They arise in longitudinal rows in the hyaline bands which follow the myoneme system closely (fig. C, *cil.*). Each cilium arises from a single basal granule which is located in the alveolar layer just below the myonemes.

Toward the posterior region the cilia and basal granules cannot be traced (fig. B). Anteriorly, the cilia are slightly longer than those of the middle region. The somatic cilia on the left side of the ventral surface form a special field in the region of the ciliary membrane and are distinguishable by their length only. They are not connected to that organ and constitute a separate, although functionally related, ciliary field which may be called the *lateral ciliary field* (fig. B, *l.c.f.*), described by Fauré-Fremiet (1924). These cilia function as subsidiary organs to the ciliary membrane.

I have been unable to relate the somatic cilia either directly or indirectly to the motorium. They follow the myonemes closely and are parallel to them. Whether or not they form connections anteriorly with the adoral neuromotor fiber I am not certain.

The *fibrillar system* is composed chiefly of the *adoral, dorsal,* and *ventral fibers,* and the *circumoesophageal ring.* The *adoral fiber* is a large granular fiber extending from the motorium around the peristome at the inner base of the collar (fig. D, *ad.f.*). It connects with the adoral membranelles, the oral membranelles, and the ciliary membrane. It is the principal fiber trunk of the neuromotor system. That the myonemes and somatic cilia connect with it is not clear from any of my preparations. The presence of twenty-two ciliary lines and twenty-two membranelles is suggestive of such connection. The small dimensions of *Tintinnopsis* prevent the determination of these connections. At the point of junction between the adoral fiber and each membranelle there are three basal bodies, described above in connection with the adoral membranelles. There is considerable evidence that the adoral fiber is not homogeneous in structure; its granular structure and its probable differential activity may be taken as evidences of this heterogeneity. Besides the adoral fiber, there are two *dorsal fibers* (fig. D, *dor.f.*) extending in the ectoplasm adorally. They end freely and fade out as they progress. A *ventral fiber* (fig. B, D, *v.f.*) extends downward from the motorium ending freely in the superficial ectoplasm. It may be homologous with the fibers passing toward the micronucleus in *Diplodinium* (Sharp, 1914).

The most interesting of the fibers is the *circumoesophageal* fiber or *ring* (figs. B, D, *ring.*). It connects with the motorium by a fiber. The circumoesophageal ring surrounds the gullet completely, exactly as analogous rings extend about the gullet in lower worms, and compares with the same structure in *Diplodinium* (Sharp, 1914). Short fibers from the circumoesophageal ring project below, surrounding the gullet.

The *neuromotorium* (fig. B, *mot.*) is the central, massive body from which the fiber system described in the previous paragraph arises. It is located in the ectoplasm of the ventral wall of the column (fig. D, *mot.*) at a point rather above the middle of the length of the gullet. In form it is cigar-shaped and dorsoventrally flattened, the ventral fiber springing from its lower end and the two dorsal fibers from the upper end. The adoral fiber arises below the dorsal fibers.

The motorium, circumoesophageal ring, and adoral fiber are differentiated with iron-haematoxylin in favorable whole-mounts. With Mallory's connective tissue stain the motorium and ring are stained red. The ring has not been recorded or figured by any previous investigator of the Tintinnoina.

The evidence that the system of fibers and the central body really constitute a neuromotor system rests upon the staining capacity, integration, and finally and most conclusively, upon experimental grounds that do not need to be reviewed again here (Sharp, 1914; Yocum, 1918; Taylor, 1920; Rees, 1922; McDonald, 1922). To the evidences already recited by these investigators, I can add little. The ciliary membrane, which is attached to the upper part of the length of the ventral body wall, is an organ the activity of which is displayed in the building and repair of the lorica. This organ is a part of the neuromotor apparatus as I have shown. Its actions are only explainable upon the assumption of intimate, functional, integrated union with the central apparatus. This connection I take to mean that the neuromotor system acts toward the accomplishment of specific ends. Feeding, locomotor, and building operations are carried out by neuromotor organs.

LIVING ANIMAL

Observations upon the living animal are difficult. Its small size, opaque house, and dense agglomeration of foreign bodies prevent the larger part of the organism from being clearly seen. At the most only the upper half or less, about the adoral region, is all that can be made out. The rapid movements of the animals are a distinct hindrance to observation. It is very difficult to keep them alive for any length of time in the laboratory. Most of my observations were carried out at La Jolla, during the summer of 1925, where conditions for the capture and observation of living material were more satisfactory than in Berkeley.

Locomotion is effected either forward or backward something after the fashion of a cephalopod mollusk. The usual direction of progression is backward, the fundus of the lorica leading and the membranelles acting as propellers. Locomotion is usually jerky or darting; sudden starts alternating with short periods of rest. If observed under a binocular dissecting microscope, in a shallow dish of water, the animals will be seen to swim about in an agitated manner, striking first the surface film and then, with a sudden motor-reaction, reversing the direction of locomotion and descending rapidly to the bottom. This reaction is often carried out regularly and rapidly until the disintegration of the animal. If animals are resting quietly upon a frond of alga or upon the bottom of the dish, they may be observed to travel with the long axis parallel with the direction of locomotion, rotating in a manner comparable to the normal locomotion of other heterotrichous ciliates (as *Stentor*), the adoral membranelles acting by a lash-like stroke. In this case the oral end leads the body. A zigzagging course is common in a free-swimming animal.

The animal may freely rotate about within the lorica. In a resting animal, the anterior end is pushed in and out of the lorica frequently, the whole membranelle system being easily and completely withdrawn. The living animal may break away completely from the lorica and swim free. Whether such animals are ever able to form a new shell or to enter a deserted one, has not been determined. The number of empty loricas found in any plankton catch is usually greater than the number containing active animals. This condition probably arises from the readiness of the animal to react to an unfavorable condition by freeing itself from its shell. These empty loricas may represent the reaction of the animal to the crowded and unusual conditions of the plankton-bucket, teeming with living matter. The rigid loricas persist for a long time.

Behind a free-swimming animal a trail of slime is usually evident. Food may become entangled in it and may be ingested when the animal rests upon a substratum.

There is a marked tendency toward gregariousness in *Tintinnopsis*. If a number are present in a dish they will be seen to gather together in a short time. In collecting, I have frequently noted that they occur almost in swarms, each individual dancing in the swarm as midges dance about flowers on a warm summer day. This phenomenon is possibly a chemotropism. It appears to be independent of light.

I have been unable to follow the behavior of living, dividing animals. In numerous instances the new adoral zone could be determined and its actions observed.

The somatic cilia beat toward the oral end of the animal in a uniform manner. These short cilia are difficult to see, but it may be determined, by a careful examination of animals which have been slowed down, that they carry detritus and faecal pellets out of the lorica and discharge them through the collar. These cilia may reverse their motions and beat backward, that is, from the oral end toward the stalk. This may be seen by watching bits of carmine passing along the column. This unusual manner of carrying in bits of matter may account, in part, for the storage of detritus in the fundus of the lorica.

The work of the ciliary membrane is important. These long cilia are united in the living animal to form a sheet in such a fashion that the membrane acts as a unit. Its motions are undulating and seem usually to have their origin as a tremor posteriorly which slowly, and then more rapidly, travels toward the peristome collar. If a bit of carmine becomes attached to it, it may be seen to be picked up and passed from cilium to cilium inward toward the peristome, where the inner row of cilia may pick it up and pass it toward the infundibulum. Its further fate cannot be traced. In locomotion, the ciliary membrane may be either held out rather rigidly, acting something like a fin disposed ventrally, or it may be in active motion and serve as an accessory locomotor organ. With its aid the animal may not only rotate itself, but the shell also. The usual function of the ciliary membrane is, however, neither locomotor nor food-gathering. It is the chief neuromotor organ, aside from the membranelles of the adoral zone, connected with the repair and building of the lorica. Bits of faeces from the fundus of the lorica are picked up and passed outward, or secreted material and parts of diatoms are brought into the lorica through its agency. Sometimes one finds the remains of diatoms or dinoflagellates (pl. 15, fig. 31) on the lorica, probably secured in this manner. Bits of matter gathered by the ciliary membrane are held within the lorica.

The active twisting and turning of the animal within the lorica probably indicates that it may free itself from its posterior attachment and become reattached (Fauré-Fremiet, 1908). A local, specialized, mucus-secreting area occupies the extreme tip of the attachment and serves to aid in holding the organism to the lorica. This area is sometimes darkly stained.

Turning to the peristome region, a striking activity seen there in the living organism is that of the oral plug. In an animal which is dying, this organ pumps rapidly up and down with a piston action. In the normal, free-swimming animal, on the contrary, the plug is rigidly held expanded. Its normal function is difficult to determine. It may serve as a closing organ as the oral plug does in *Balantidium* (McDonald, 1922), or it may aid in feeding; by pushing action it may serve to crowd large dinoflagellates and diatoms down the rather narrow, but widely extensible, gullet.

The tentaculoids are extensible, claviform, or stalked, globular organs surrounding the peristome collar. They may be seen to expand and contract readily. Trichocysts are present on their globular, distal surfaces. It is very difficult to witness the action of the accessory combs in a living animal. They may be accessory feeding organs. The contractile tentaculoids may be freely withdrawn into the substance of the combs since these two organs are structurally related (fig. D, *ac.c.*, *tent.*).

The adoral ciliary crown of membranelles waves back and forth with a slow motion. Waves of contraction are initiated at the base and pass outward along the outer edges, the inner cilia moving secondarily. At a point about two-thirds above their origin on the collar, the inner cilia seem to break free from the sheathing and beat freely. They then act semi-independently. The membranelles may act together in a coordinated manner or they may act independently of each other, especially when in contact with a substratum or in feeding. The motion of the free membranelle is so directed that the effective stroke is from inward outward. When the direction of locomotion is reversed, the animal may reverse the direction of the effective stroke. Usually the avoiding of an obstacle in the pathway of a moving *Tintinnopsis* is accomplished by a reversal of the direction of locomotion and by a rotation of the body; the animal bores its way through the medium.

In the oral membranelles, undulating waves of beating pass from a point of origin toward the mouth in such a manner that the effective stroke is directed inward. The oral membranelles may beat constantly, even when the animal is contracted within the lorica. Their power of organizing small particles into food boluses is obvious.

DIVISION

The division of *Tintinnopsis nucula* has not been the subject of previous special study. Entz, Jr. (1909) and Schweyer (1909) have discussed the results of their observations upon various species of Tintinnoina. My account differs so radically from that of these writers that I shall treat it in special detail.

Division stages are obtainable in animals collected at almost any time during the day. Most division phases seem, however, to occur in the early hours of the morning. In plankton catches made during the early morning, from two to nine o'clock, almost all phases are present. These results are comparable to those obtained in the dinoflagellates (Hall, 1925 *a, b*). They demonstrate that the rhythm of fission is part of the cyclic ebb and flow in the diurnal pulse of living matter; this rhythm is not confined to this group of protozoan organisms.

CYTOSOME

The earliest steps in preparation for division are seen in the cytosome rather than in the nuclei. The membranelles are formed and the new cytosome is almost completely developed before the nuclei have undergone visible changes or have shifted their position. During the whole period of binary fission both nuclei and cytoplasm act together toward the accomplishment of the common end.

It is very noticeable that the early stages of fission are abundant, and frequently in a plankton catch almost every animal is in some early phase of division. The late stages are few in all cases. It is probable that these early stages require a long period for their development and the final stages are rapidly completed.

The beginnings of division in the cytosome are to be looked for on the ventral side in a position midway between the area marked by the macronuclei. The earliest stage that I have been able to see is indicated by a line in the pellicle (pl. 13, fig. 13). This line, which is short and just below the end of the ciliary membrane, is distinctly marked as a transverse clear area below which a row of basal granules soon appears. The line crosses the level of the midpoint of the macronuclei. Slightly later the right end of this line starts to curl inward. Its left end starts to grow upward. As the line curls upward its spiral course becomes rather tightly wound (pl. 13, figs. 14, 15). When this condition is reached short cilia arise from the basal granules. The cilia soon grow long and assume the appearance of short adult membranelles. Shortly before they have taken on this structure, the cytoplasm surrounding the newly differentiating cytosome becomes massed about it so that the posterior daughter forms a lateral bud on the cytosome (pl. 14, fig. 19). This bud grows rapidly until it takes over the whole mass of the posterior protoplasm of the organism (pl. 15, figs. 21, 25).

Entz, Jr. (1909), claims that the basal bodies of the adoral membranelles arise in connection with either the macronucleus or the micronucleus. They are said to arise as granules emitted from the nucleus, passing through the cytoplasm in a definite manner and finally becoming deposited about the newly forming peristome. Hartmann and Schilling (1917), apparently without actual observations, criticized this view, asserting that it remains an unconfirmed and unique case. My observations both upon whole mounts and sections sustain this criticism. In no case have I been able to trace granules from any nucleus to the outer wall in the asserted definite course; the granules described by Entz, Jr. (1909), are possibly parasites. Maier (1903) considers that the basal granules arise as local cytoplasmic bodies at the surface of the pellicle. No evidence regarding this view can be demonstrated at this time in my materials. It is probable that the basal bodies arise by outgrowth in connection with the newly forming neuromotor apparatus, although conclusive evidence is yet lacking.

When the posterior zooid has reached the stage described in the preceding paragraph, the anterior or apical zooid, together with all its parts which occupy the upper half, elongates and rapidly assumes the appearance of a typical *Tintinnopsis*. This elongation continues rapidly until the animal is composed of two zooids (pl. 15, fig. 25) each functioning separately, but connected by a thin, short strand of protoplasm. I cannot come to any satisfactory conclusion regarding the nature of the fission line. It is produced as a white line not unlike that in *Stentor* (Johnson, 1893). The forces which operate to effect the separation of the two zooids are probably internal and involve changes in sol-gel reversible protoplasm. I am confident that the myonemes are not cut at first by the fission line but simply become bent sharply inward. Until a very late stage in division they are accurately matched on either side of the fission line, although the stripes are somewhat narrower at the posterior end of the distal zooid. Later changes finally cut the myonemes apart as the fission line widens and deepens. When finally freed the two daughters are each provided with a lorica.

The fission stages described above resemble closely those of *Stentor* (Johnson, 1893). They represent a mode of division probably common in the strongly polarized heterotrichous ciliates.

The external changes in *Tintinnopsis* described above are accompanied by internal changes and reorganization. Profound changes in viscosity are evident. The cytoplasm becomes extremely fluid. This is evinced by the swinging of the macronuclei into a characteristic position (pls. 13, 14). These currents may be compared with the cytoplasmic streaming of cleaving metazoan eggs (Chambers, 1924). The modifications in viscosity, depending upon a probable solation of the cytoplasm, appear to be regular. It is probable that they are evidences of a regular physiological and general reorganization of the whole organism at this critical period of binary fission.

Rapid changes in growth and in lengthening of the anterior zooid result in its physiological isolation from the newly formed one. This isolation probably results in the independent further differentiation of the new cytosome. Physiological isolation may stimulate the rise of parts in certain cases, especially in the lower worms and in some protozoans, notably *Stentor* (Child, 1915).

I have been unable to trace any dedifferentiation and redifferentiation in the ciliary zones of the anterior daughter zooid comparable to that in the Hypotricha and Holotricha (Yocum, 1918; Rees, 1922) during binary fission. The fission of *Tintinnopsis* resembles that of *Stentor* (Johnson, 1893).

Agamic reproduction or binary fission in the ciliates is a process closely resembling the fission of metazoans such as *Planaria* or *Nais*, the strobilization of Scyphozoa, or the formation of gemmules in sponges and Bryozoa. The binary fission of *Tintinnopsis* is to be regarded as allied to this type of reproduction of functional individuals, each with a similar genetic pattern (Kofoid, 1923).

NUCLEI

Nuclear and cytoplasmic changes are nearly simultaneous. The micronuclei generally complete their division before the macronuclei have formed a reconstruction zone. The micronuclei behave alike and usually begin their divisions about the same time although they are neither synchronized nor mutually dependent throughout mitosis. A description of the behavior of one will apply equally to the other.

The interkinetic micronucleus is a spherical or subspherical, granular, and rather faintly staining body, already described. During the first part of the prophase it moves away from the interior polar macronuclear depression in which it is frequently lodged. It moves toward the level of the middle of the macronucleus and while so doing swells and increases greatly in size, from two or three microns to nearly five microns in diameter (pl. 15, fig. 33). It becomes more granular. The granules which compose this mass become aggregated into the central portion of the micronucleus, leaving the margins clear (pl. 15, fig. 34). It is very difficult to see in this small nucleus any organization into a spireme. Shortly after the granules of the micronucleus have become aggregated into this central mass the nuclear wall shows signs of a slight elongation (pl. 15, fig. 35). The polar caps form thickened crescents about the two ends of the forming spindle and the chromatin granules become distributed along the spindle fibers. These granules may now be called chromosomes (pl. 15, fig. 36). The course of events at this point is not clear but as far as can be made out from preparations it seems that these chromosome threads become massed into a metaphase clumping in the center of the spindle, leaving the area toward the ends of the polar caps clear and free (pl. 15, fig. 37). In the anaphase the chromosomes become separated into two groups, one at either end of the now elongate nucleus, massing densely about the polar cap (pl. 15, fig. 29). In the final phase the two halves of the nucleus pinch away from each other until they are connected only by a delicate strand of the nuclear membrane (pl. 15, fig. 27) which later breaks and frees the two daughters. The internal change which accompanies these telophase changes is a progressive break-up of the chromosomes into granules which are at first crowded about the pole of the nucleus and which later form a loose cloud within the nucleus (pl. 15, fig. 27).

At no time during the course of this mitotic process is it possible to accurately estimate the number of chromosomes. Their number is certainly great, possibly more than a hundred, and their small size, combined with irregularity of linear structure, prevents an accurate counting. I am unable to determine the nature of the division of the chromosomes.

As is the case with the micronuclei, the macronuclei divide nearly simultaneously. A description of events in one macronucleus will apply also to the other. The structure of the resting nucleus has been already given in a former section and need not be repeated.

The first indication of division in the macronucleus is the formation of a clear zone transversely across the center (pl. 15, fig. 23). Usually this clear zone is exactly across the center but sometimes it separates the two parts of the nucleus into slightly unequal portions. No changes in shape are at first associated with these changes. When the clear zone is complete and there is no indication of cloudiness within it, the nuclear wall constricts in such a manner that the nucleus assumes a dumbbell shape. This constriction narrows and pinches in until the nucleus becomes finally divided in two (pl. 15, fig. 24).

The clear zone or reconstruction band is without structural differentiation. It is the same as the "*Kernspalt*" of other ciliates. It is formed in *Tintinnopsis* by a migration of the chromatin substance away from this region of the macronucleus. As division proceeds, longitudinal strands appear on either side (pl. 15, fig. 24). These may be somewhat similar to the strand-like organization of the chromatin in the macronucleus of a dividing *Cycloposthium* (Dogiel, 1925).

At the beginning of division, the larger macrosomes within the macronucleus are scattered irregularly throughout its substance. As the process proceeds they become aggregated at either end. No evidence of their division can be made out in my material although it appears that their number is nearly constant (Hegner and Holmes, 1923). They may represent trophic chromosomes. The smaller granules are aggregated simply at either end of the cleaving nucleus. The nuclear wall does not break down at any time during the normal process of division. Vacuoles surrounding larger granules are sometimes evident. The newly formed macronucleus of *Tintinnopsis* is about one-half the size of the one from which it came. It grows rapidly and in the period just before the separation of the daughter animals has assumed normal size.

A regular and constant series of changes in position is assumed by the macronuclei in division. These are probably conditioned by the streaming and changes in viscosity of the cytoplasm during this exceedingly plastic period. The two macronuclei occupy the middle region of the animal and are about equidistant during the trophic period. By the time the reconstruction band appears they have migrated in such a manner that their inner, concave sides form an almost complete circle about the ventral surface which is to be the site of the newly forming, daughter cytosome (pl. 12, figs. 7, 9). They approach gradually and by the time the daughter cytosome is almost fully formed they are in close approximation. They then divide; the adoral halves of the macronuclei are retained by the anterior animal and the posterior halves move into the cytoplasm surrounding the (posterior) daughter cytosome (pl. 15, fig. 21).

During the whole period of nuclear changes described above, there are no indications of nuclear conjugation (Laackmann, 1906) either of the micronuclei or of the macronuclei. The process of nuclear division in *Tintinnopsis nucula* is comparable to the nuclear behavior of other ciliates.

LORICA

The fate of the lorica during binary fission is interesting in that the parent lorica is inherited. In a dividing pair of zooids, the anterior (oral) animal first secretes a delicate, transparent, irregular, foam-like substance from its protoplasm, which is at first slightly viscous and easily picks up the stored faecal reserves from within the fundus of the parent lorica. This mass is then passed anteriorly by the action of the ciliary membrane of the posterior zooid (pl. 15, figs. 28, 31). This foreign matter, excreta and foamy secretion, is firmly cemented together and forms the new shell. The matrix rapidly and progressively hardens into the characteristic architecture of the lorica. Thus in large part the workmanship of the construction, especially of the posterior end, falls upon the posterior member which itself acquires the old lorica at the final separation of the two zooids. From time to time the animal within a lorica may add to its own lorica by its own neuromotor activities.

The sources of the matrix are to be found in the deposition of materials from the animal. This matter may be (1) a general secretion from the ectoplasm of the animal of the colloidal substance which hardens into the prismatic elements. For this there is no evidence in *Tintinnopsis nucula.* However, in *Tintinnidium primitivum* [*nomen nudum*] Busch (1925) reports this to be the case, the secretion arising from the peripheral vacuoles over the general surface. (2) Material poured out from the mouth or from the anus and stored as faecal pellets and as frothy matter within the lorica, often in sufficient amount to account for the material forming the shell, or (3) a local secretion from the labile and active lateral lobe. The evidences for the second source are the extraordinary metabolic activity of the animal, the large vacuoles which crowd the cytosome, and the well-developed cytopyge. The evidence which favors the third view is to be found in the staining of the lateral organ. It is sometimes deeply stained with haematoxylin, especially in a dividing pair, and this dark siderophile condition (fig. B, *lobe*) may be taken as evidence of secretory activity. The lateral lobe is in a position to shape up chiefly the smooth interior lamina of the lorica, and the ciliary membrane, the outer reticulated surface. Kofoid (1905) suggests: "The type of shell structure in *C. fasciata* suggests the slow rotation of the animal in a constant direction during the deposition of the shell-forming substance and the location and limitation of the region of its extrusion to a single place upon the animal."

Not only secretion but also *activities are principal agents in the assemblage of the house* in these forms. The substrate arising from the lateral lobe and the stored reserve are profoundly affected by sterotyped neuromotor behavior. While secretion determines the material of the lorical pattern, behavior moulds its characteristic architecture, unlike the shells formed in larger part by secretion in some rhizopods, or possibly wholly by secretion in the internal skeleton of radiolarians. The ciliary membrane, lateral ciliary field, adoral membranelles, and the lateral lobe act together in a coordinated manner in the building of the shell, using the faecal matter stored within the parent lorica together with a secreted substratum and adding to these extraneous matter gathered from outside by the animal. Thus *the construction of the lorica becomes a matter of neuromotor activity, through the cooperation of several organs toward the accomplishment of a common end.*

Kofoid (1905) stressed the relationship between the number of adoral membranelles and the serrate margin of the lorica in *Tintinnus serratus.* In describing *Tintinnopsis reflexa* he remarks: "The significance of this limitation in proportions lies, it seems, in the dependence of this projecting portion of the shell upon the *length of the cilia and intercalary cirri of the adoral plates.*" Kofoid (1905) was unacquainted with the ciliary membrane, which appears in *Tintinnopsis nucula* to be one of the principal neuromotor agents acting in this function of house-building. Some of the tintinnids unprovided with a ciliary membrane, examples of which are *Tintinnus lusus-undae, Cyttarocylis serratus,* and *C. ehrenbergii,* do not agglutinate foreign bodies nor do they build them into their permanent structure.

The slight spiral structure of the collar of the lorica is evidently the morphological expression of the leiotropic disposition of the shell-forming organs. While *Tintinnopsis nucula* does not clearly show evidence of spiral structure in the body of the lorica, that tendency is found to be present or even pronounced in other tintinnids, as in *Cyttarocylis fasciata* (Kofoid, 1905).

In binary fission the posterior daughter zooid acquires the old lorica. The older peristome and its organs, which belong to the anterior daughter zooid, have had secreted and built up for them a new lorica by the progressive and joint action of the parent and the offspring. This condition is different from that of certain dinoflagellates (Kofoid, 1908) in which part of the skeletal plates are passed to each daughter, and from the ciliate *Coleps* in which conditions comparable to those in dinoflagellates occur. In the case of *Tintinnopsis,* however, the shell is passed *in toto* to one daughter and a wholly new one formed for the freed daughter.

PARASITES AND DEGENERATION

Parasites have been known for some time to inhabit various tintinnids. Most of these are described as voluminous cytoplasmic inclusions which by their growth ultimately disrupt the organism (Laackmann, 1906; Duboscq and Collin, 1910). These are supposed to be parasitic dinoflagellates and are comparable with the various other parasitic members of that group described by Chatton (1906). So far as I have been able to determine there are no other parasites described from tintinnids.

I have encountered such voluminous cell inclusions in only two or three cases. In all these cases the inclusion occupied the posterior part of the organism.

In a great many instances, especially in the plankton collections during the late summer and early fall months, dividing *Tintinnopsis* revealed, when stained and mounted, abnormal nuclear conditions which so resembled the stages of nuclear conjugation described by Laackmann (1906) that I was led to make a special study of them. In some collections nearly every animal was in some stage or other of division associated with disintegrating nuclei (pl. 13, figs. 10, 12). These disintegrating nuclei were in all cases distinctly filled with the large granules. Many of these were surrounded by distinct vacuoles. That these vacuoles indicate solution of the nuclear contents is evident.

Thin sections, carefully differentiated after staining in Heidenhain's iron-haematoxylin, revealed the structure of the granules surrounded by a vacuole. In each there is a clear, non-staining, central area in the midst of which a single intensely staining granule is distinctly visible (pl. 13, fig. 11 *a*). In certain cases examined carefully, it was found that the central granule had divided into two parts which were connected by a thin, deeply stained, connecting fiber (pl. 13, fig. 11 *b*). In other cases, still more rare, the central granule was divided into four parts in a cross (pl. 13, fig. 11 *c*).

Numerous instances of the escape of these bodies into the cytoplasm have been observed (fig. F, *pa.*). The nuclear wall in these cases is partially disintegrated and broken down. The parasites are discharged freely into the cytoplasm, and are carried about in such a manner that they form a loose cloud-like mass about the region of the newly forming cytosome (pl. 13, fig. 12). There is considerable evidence from the examination of many of these parasitized *Tintinnopsis* that the parasitic infection, possibly accompanied by the extrusion of chromatin into the cytoplasm, so stimulates the animal that plasmotomy is initiated. In no case, among hundreds of individuals, was division ever carried out beyond the spiral inrolling of the ciliated daughter peristome. The animals which reach this stage are usually, but not always, incapable of further development and degenerate and disintegrate. The parasites increase in size in the cytoplasm after being freed from the nucleus. I cannot connect them with the voluminous inclusions or dinoflagellates described above.

The loose cloud-like mass distributed about the daughter peristome is the chromidial mass or cloud which I described in a previous section.

Its appearance is comparable to that figured by Entz, Jr. (1909, pl. 19, figs. 1–9, and pl. 18, figs. 1–2), for *Cyttarocylis,* and asserted to be present in other Tintinnoina. In some cases, although rarely, it is present in an animal the nuclei of which are hardly abnormal. In these cases, the parasites may have been carried over in the cytoplasm by a recently divided infected animal and await there opportunity to infect the nuclei of the animal. Such instances might be compared with an incubation period. In a few instances, carefully differentiated, lightly stained whole mounts of some animals revealed the "nucleated" parasites exactly as they are found in sections (pl. 13, fig. 12). Usually they stain homogeneously and then their appearance, together with their regular distribution to the region of active cytoplasmic changes, has undoubtedly misled Laackmann (1906) and his follower (Merkle, 1909) to the erroneous conclusion that they were actually *chromidia* and essential to the normal reproduction of Tintinnoina. I believe that this erroneous conclusion is a confusion of chromidia

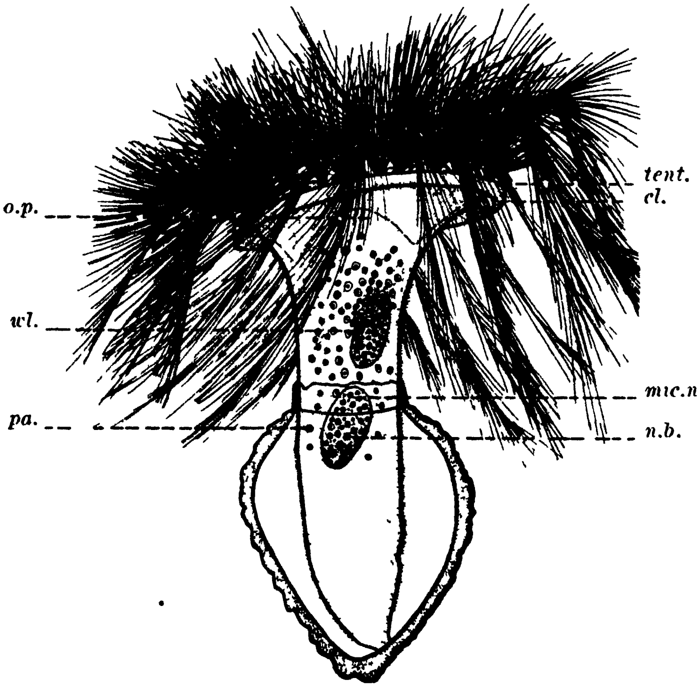

Fig. F. Semidiagrammatic figure of *Tintinnopsis* showing cytoplasmic and nuclear phases of *Karyoclastis tintinni* gen. nov., sp. nov., within the cytosome. Abbreviations: *cl.*, peristomal collar; *mic.n.*, micronucleus; *n.b.*, trophic chromosomes within the macronucleus; *o.p.*, oral plug; *pa.*, parasitic *Karyoclastis; tent.*, tentaculoids; *wl.*, nuclear wall. × 850.

with these parasitic organisms. That some chromidia, if properly and carefully stained, will reveal themselves as parasites in many Protozoa has been recently expressed by Kofoid (1921).

These parasitic bodies may possibly inhabit but one nucleus and cause its destruction only (fig. G) or they may attack both nuclei simultaneously (pl. 13, fig. 10).

These parasites are distinct in structure in many respects from *Nucleophaga* of fresh-water Protozoa (Dangeard, 1895) and from *Sphaerita* from the intestinal amoebas. For this reason I shall propose for their reception a new and provisional genus, *Karyoclastis.*

Karyoclastis gen. nov.

Intracellular parasite, primarily intranuclear but with a cytoplasmic phase. Ovoid, globular, or irregular in outline. Individuals separate. An outer mantle, possibly cytoplasm, staining gray in iron-haematoxylin, surrounds an inner clear area in the midst of which is a single black-staining granule. Undergoes binary and repeated fission by the division of the central grain. Division in nucleus of host causes a dissolution and vacuolization of nuclear substance so that the individuals are set free into the cytoplasm. Unknown relations and uncertain position.

Karyoclastis tintinni sp. nov.

Karyoclastis inhabiting various marine members of the Tintinnoina. Having alternating cyclic phases within the nuclei and the cytoplasm. Spores set free by the disintegration of the host or carried along by binary fission from host to host, possibly stimulated by parasitism. Morphological characters identical with those of the genus. Host, *Tintinnopsis nucula,* from San Francisco Bay.

Among the *Tintinnopsis* collected are certain rare forms which may be degenerate forms (pl. 15, figs. 26, 30). The adoral zone is lost and the organism becomes contracted into the fundus of the lorica. In one or two cases (pl. 15, fig. 32) the animal was freed from the lorica. These forms may represent either of the following conditions, (1) degenerations due to parasitic infection, (2) normal phases in the life-cycle. The first seems probable since in these forms the nuclei are usually abnormal and do not present the condition of a normal process. Parasites are also usually present in them.

I have not seen any forms of *Tintinnopsis nucula* which might be designated as resting cysts (*Dauercysten*). In *Cyttarocylis* such cysts are reported (Busch, 1919). They are surrounded by a hard cyst wall and do not resemble the degenerate forms which I have described above.

Endogenous budding or sporulation does not occur in *Tintinnopsis* so far as I have been able to determine. Nearly all cases of reported endogenous budding (Walker, 1909) have proved to be either cannibalism (McDonald, 1922) or due to ingested foreign bodies. In a few cases I have seen within the cytoplasm small dinoflagellates which might have been mistaken for spores or internal buds. Wailes (1925, pl. 2, fig. 18) has reported spores in *Tintinnus subulatus* Ehr.

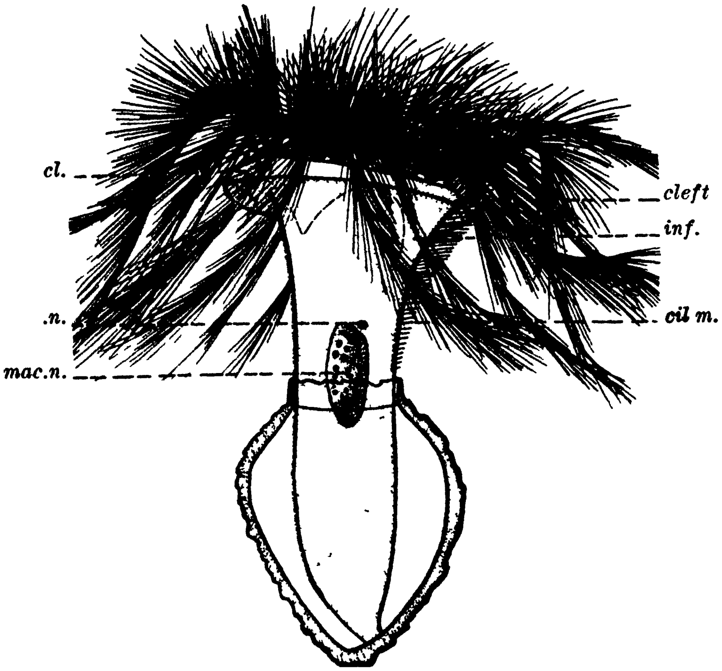

Fig. G. Semidiagrammatic sketch of *Tintinnopsis*. Form with a single macronucleus. Abbreviations: *cleft*, cleft in peristome; *cil.m.*, ciliary membrane; *cl.*, peristome collar; *inf.*, infundibulum; *mac.n.*, macronucleus; *mic.n.*, micronucleus. × 850.

Forms of *Tintinnopsis campanula* with but a single round or ovoid macronucleus and a single micronucleus are described by Laackmann (1906). They were designated by him as *"Jungenformen"* in the belief that they represented the products of macrospores and microspores which had conjugated. The youngest of these were supposed to be devoid of the adoral membranelle zone.

I have occasionally seen forms of this nature (fig. G, *mac.n.*) in *Tintinnopsis nucula.* They are not limited to the spring plankton but are scattered throughout the year. In all, there is no evidence of growth in the adoral zone. They are normal save in having a single macronucleus. In several cases there were a single macronucleus and two micronuclei. In some cases these forms may be the result of the parasitism described previously or in others they may represent ex-conjugants and the reorganization after the period of sexual reproduction. The lack of conclusive evidence regarding the so-called conjugating macrospores and microspores and the hypothetical resting stage in the depths of the sea (Laackmann, 1906) make my interpretations of a parasitic origin of the irregularities more plausible. Parasites are frequent in the cytoplasm and nuclei of these forms. The irregularities probably represent the results of differential parasitism and partial recovery. Conjugation of individuals of the usual type is known to occur (Bresslau, 1906) in these forms and will account for the most of the biological phenomena which this hypothesis attempts to explain. No evidence regarding hypothetical macrospores and microspores has been noted in my preparations. Inclusions that might be so regarded have always proved upon careful treatment to be food bodies, usually ingested dinoflagellates.

DISCUSSION

No account has appeared describing a neuromotor system of any member of the genus *Tintinnopsis,* or indeed of any tintinnid. In this paper there are described for the first time, a complex neuromotor system, the labial lateral lobe, the cleft of the peristome collar relating to the spiral organization of the adoral zone, as well as other minor features. An extended account of the structure and physiology of the ciliary membrane is given and its relations to house-building shown. An account of binary fission is offered which places *Tintinnopsis,* and probably the whole group of tintinnids, within the bounds of what we know regarding other ciliates.

The only previous mention of the ciliary membrane is that of Fauré-Fremiet (1924) who failed to follow the course of this organ into the peristome and was unable to describe its functions. The mobile lobe which plays an active part in the building of the lorica has not been previously noted in *Tintinnopsis nucula.* The ciliary membrane finds no exact parallel in other heterotrichous ciliates without a lorica.

Tintinnopsis nucula possesses a complex neuromotor apparatus. This organ system comprises every motile organ of the body. The coordinating center or neuromotorium constitutes the directing center. It is located in the ventral ectoplasm. In form it is spindle-shaped or cigar-shaped with a ventral fiber, two dorsal fibers possibly connecting with the striated surface of the peristome plug, and a powerful adoral fiber which connects with the basal granules of the adoral membranelles, oral membranelles, and the ciliary membrane. A special fiber connects the circumoesophageal ring. This neuromotor system compares favorably with similar systems in other ciliates. In many respects it is one of the most centralized and compact neuromotor systems yet described in any ciliate. In centralization it stands in close association with the neuromotor system of *Diplodinium*. Both *Diplodinium* and *Tintinnopsis* are alike in the possession of a central motorium connecting a circumoesophageal fiber and with adoral fibers connecting the adoral membranelle zones. The neuromotor system of *Paramecium* (Rees, 1922), *Balantidium* (McDonald, 1922), and *Euplotes* (Yocum, 1918) are comparable in that they consist of fibers which are distributed to the motor organs that have their origin in a common motorium-mass and, in some, the existence of a neuromotor fiber related to the adoral zone.

As far as the neuromotor system of the ciliates has been carefully examined, it is composed of a single central mass located near the gullet which sends out fibers to the motor and feeding organs integrating them structurally. Taylor's experimental studies upon *Euplotes* (Taylor, 1920) have conclusively demonstrated the assumption of previous writers that this system of converging intracytoplasmic fibers really represents a system of organs integrative of the neuromotor activities of these ciliates. That the neuromotor system actually integrates the activities other than locomotor of ciliates was forecast in *Folliculina* (Andrews, 1920) in the following words: "Were there a complex nervous system we might refer much of these activities *of house-building* (italics mine) to it." In *Tintinnopsis nucula* such a neuromotor system is actually described and its part as the active agent in house-building is shown.

Yocum (1918) aptly points out the inherent weakness in the idea of the neuromotor function of the neurophanes described in some ciliates, and Entz, Jr. (1909), was unable to discover any evidences of these structures in Tintinnoina, nor have I been able to do so.

The protozoan individual may be unicellular or it may be multicellular. In the hypermastigote *Trichonympha,* Kofoid and Swezy (1919) state that "the organism and not its cells is the fundamental basis of differentiation." *Trichonympha,* it must be confessed, is a unicellular protozoan. However, this organism displays a complexity and integration of organs almost as structurally diverse and functionally able as its metazoan termite host. In *Proboscidiella* (Kofoid and Swezy, 1925) the organism is controlled by a variable number of nuclei. These hypermastigote flagellates display within themselves all stages of transition from the unicellular individual to that of the multicellular. In *Tintinnopsis nucula* the individual is multicellular. Its four nuclei are not a pair of discrete amphinuclei. Each nucleus of *Tintinnopsis* has its own behavior, divides independently, and finally is passed on to the two new daughters at the time of binary fission as a distinct, separate, independent unit. Dobell (1911, 1925) affirms that protozoan individuals such as *Tintinnopsis* are non-cellular; this author would claim that the criterion of cellularity resides not within the cytoplasm and its organ the nucleus but rather in the cell wall. The noted English protozoologist focuses his attention upon the cell wall and not on the contents. A cell is a "mass of cytoplasm surrounding a nucleus" (Wilson, 1925). Cells are interdependent masses of the living substance, protoplasm, organized into contrasting, changing systems. We may recall in this connection that in the annelid *Chaetopterus* experimental results have been obtained which demonstrate that differentiation may occur without cytoplasmic cleavage (Lillie, 1902). In the tapeworm, to cite another metazoan, the greater mass of the adult is syncytial, yet it cannot be stated that the organism is undifferentiated in any degree, or that it lacks cells. *Tintinnopsis* is a four-celled somatella. It may, however, under unusual conditions, contain but two nuclei (fig. G) or it may contain sixteen in the period of conjugation (Bresslau, 1906). The whole complex of organization and its visible, morphological expression in the differentiated somatella is the fundamental basis of organization in *Tintinnopsis.* This ciliate is not only cellular but it is also equally multicellular. Hardly an hypothesis applied to the Protozoa has been more misleading than the concept of them as simple unicellular organisms which are not subject to the same essential laws of organization and behavior as the more prominent forms of life with which we commonly deal (Kofoid, 1923); the cell remains, however, as the arena of evolution.

The chromidial hypothesis (Hertwig, 1902) cannot apply to *Tintinnopsis.* No evidence is brought forward that the chromidia which are described by Entz, Jr. (1909), are probably other than nuclear and cytoplasmic parasites. They are not a part of the nuclear cycle. The doctrine of the individuality of nuclei expressed in the familiar aphorism *omnis nucleus e nucleo* is clearly demonstrated in the binary fission of *Tintinnopsis.* The chromidiogomy of *Polystomella* and of the protozoans in general is probably also to be attributed, in part at least, to internal parasites (Kofoid, 1921).

CONCLUSIONS

1. *Tintinnopsis nucula* is a frequently occurring marine ciliate which maintains itself within coastal waters. It is cosmopolitan in distribution and omnivorous in feeding habits.

2. *Tintinnopsis nucula* is a member of the genus *Tintinnopsis* and can be definitely placed in Brandt's (1907) definition of this genus. It agrees with Fol's (1884) description which was emended by Laackmann (1907). My material was less variable than that previously described. Measurements of the lorica show that it does not deviate more than five microns from the mean length. Its other dimensions are proportional. It is probably not a small form of *T. ventricosa,* its nearest relative.

3. No previous detailed description of the morphology of this species has been made. In this study, the ciliary membrane and the lateral lobe are described for the first time and their functions indicated. They are the chief organs concerned with the building and repair of the lorica. The peristome collar is shown to be a reflexed, spiral thickening of the margin of the column wall. Through it the ciliary membrane passes to the column. Within the peristome field the oral plug arises. The adoral membranelles are twenty-two in number; three of them send proximally into the gullet membranes which become modified to form oral membranes. The somatic cilia form a lateral field and a general somatic field. The cilia are organized in longitudinally disposed rows following the highly contractile myonemes. A stalk attaches the animal to the lorica. There are twenty-two extensible tentaculoids. From the proximal end of each tentaculoid extends an accessory comb into the peristome. The tentaculoids are armed with trichocysts. There is no contractile or non-

contractile vacuole in this species. Mitochondria are present within the cytoplasm.

4. The nuclear system of *Tintinnopsis nucula* is composed of four discrete nuclei, two of which are macronuclei and two are micronuclei. They are normally constant in number and position. The macronuclei are made up of large granules tending to be constant in number in a given individual. Additional small granules are scattered irregularly throughout the substance of the nucleus. The micronuclei are about three microns in diameter and possess a granular structure.

5. There is a complex neuromotor apparatus. The central massive motorium located on the ventral side of the column beneath the gullet may be presumed to be the directing center since it is intimately connected with most and presumably all the motile organs of the body. It is spindle-shaped. Anteriorly it gives rise to two dorsal fibers ending in the peristome and probably connecting with the striations in the ectoplasm of the oral plug. Posteriorly there is a single ventral fiber ending freely in the ectoplasm and directed toward the nuclei. The circumoesophageal ring surrounds the gullet and connects with the motorium by a fiber from its proximal end on the ventral side. The circumoesophageal ring extends fine fibrils partly down the length of the gullet. The adoral fiber extends from the motorium about the circumference of the peristome, connecting each membranelle with the others. It is the most prominent and easily distinguishable member of the neuromotor system. The myonemes constitute the contractile elements of the neuromotor system. They are twenty-two in number. They extend from the base of the animal to the margin of the peristome collar in an unbroken series and are granular in structure. Between them are the clear hyaline bands from which the cilia arise from basal bodies. A lateral field ciliary area arises from these cilia at the right of the ciliary membrane. The ciliary membrane is a structure made up of powerful, elongate cilia in a single series following a spiral course down the length of the column. It arises by a gradual change in the structure of the distal end of the adoral membranelle series. In function it is the most important organ in the building and repair of the lorica. Oral membranes are found in the gullet. These are three in number and arise by a direct transformation of the adoral series. They end in large basal bodies at the base of the gullet.

6. *Tintinnopsis nucula* is a quadrinucleate somatella. It is not only cellular but is also equally multicellular.

7. In fission, each nucleus gives rise by transverse constriction to two daughter nuclei. The division of the micronuclei is mitotic. The macronuclei contain a reasonably constant number of formed bodies which may represent trophic chromosomes. These divide and become distributed to the daughter nuclei. Linear organization of the chromatin occurs within the nuclear walls of the macronuclei. The adoral membranelle zone of the newly forming daughter animal arises posteriorly and ventrally in a constant position and the nuclei are oriented about it in a definite manner beneath it. The first sign of division is cytoplasmic and may be seen as a clear line across the myonemes which gradually spirals upon itself forming the peristome. From this line, the basal granules arise and from them the membranelles arise. Other organs appear to be formed *de novo.*

8. The lorica is retained by the posterior daughter animal of a dividing pair and is thus passed on from one generation to the next. The anterior animal of the dividing pair forms a new lorica, partly with the aid of the posterior animal. The neuromotor activities of the animal play a fundamental part in the construction of the lorica. The ciliary membrane is found to be a principal organ concerned with this function. The ciliary membrane is a neuromotor organ.

9. Viscosity changes in the cytoplasm evinced by profound movements of the nuclei during fission are evidences of the sol-gel reversible nature of protoplasm. These movements are characteristic and take a definite path which is constant.

10. Parasites inhabiting the macronuclei and responsible for abnormal degenerating forms, are described. A new genus and species of these parasites of doubtful position is erected. *Karyoclastis tintinni* is a parasite with an intranuclear and intracytoplasmic phase and with constant morphology and behavior. It undergoes binary and repeated fission within the macronucleus and is discharged, upon the dissolution of nuclear wall, into the cytoplasm. Misinterpretation of the nature of these parasites has led to the application of the chromidial hypothesis to the reproduction of tintinnids (Entz, Jr., 1909). *Karyoclastis* cannot be confused with the dinoflagellate parasites described by Chatton (1906) or with *Sporocystis* (Duboscq and Collin, 1910). It is unlike *Nucleophaga* (Dangeard, 1895) or the related *Sphaerita* from the nuclei of the intestinal amoebas. These parasites probably account for the irregular distribution of most of the forms with a single macronucleus in the plankton, and for other abnormalities.

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EXPLANATION OF PLATES

All figures drawn with camera lucida from whole mounts of preparations fixed in hot Schaudinn's fluid and stained with aqueous iron-haematoxylin. × 525 unless otherwise indicated.

PLATE 12

Fig. 1. Showing the myonemes with narrow bands between, the claviform tentaculoids, and ciliary membrane. The cytopyge is large.

Fig. 2. *Tintinnopsis* viewed from above downward. Showing the oral plug, peristome collar, gullet, and ciliary membrane. The trough through which the ciliary membrane passes is shown below. The position of the tentaculoids and membranelles is diagrammatically indicated.

Fig. 3. Shows especially the mode of attachment at the base of the lorcia. Basal granules of the ciliary membrane are to be especially noted. The nuclei of this specimen are somewhat unusual in shape.

Fig. 4. Lorica from below showing the structure, particularly the elevated, irregular fenestrations.

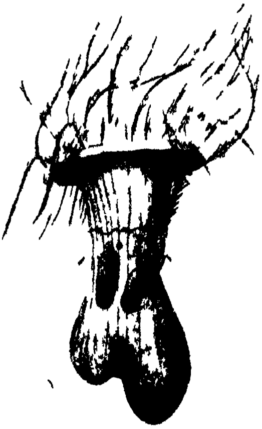
Fig. 5. A fully expanded and attached specimen. The ciliary membrane and membranelles are clear. The nuclei are normal in shape and position. The tentaculoids are heavily stained.

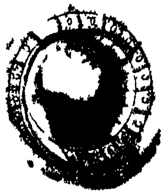
Fig. 6. Lorica from above downward showing the nuchal band, collar, and fenestrations.

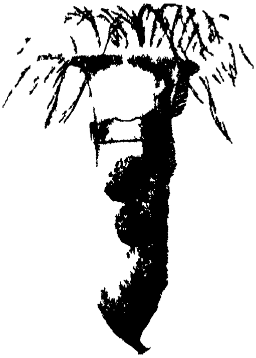
Fig. 7. The collar of this specimen is expanded but the tentaculoids are nearly contracted. The nuclei have begun to divide. The ciliary membrane and basal granules are clear. The circumoesophageal ring and motorium may be seen near the gullet, beneath the ciliary membrane.

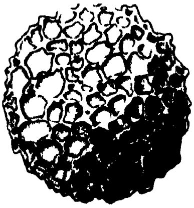
Fig. 8. Lorica from the side showing general structure, especially of the collar, nuchal band, and fenestrations.

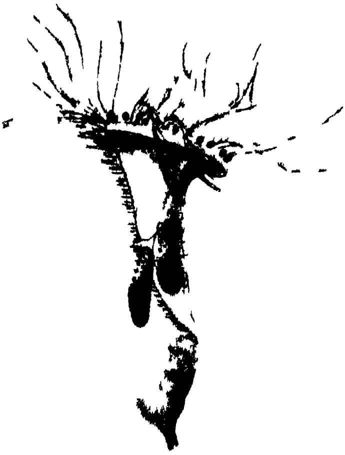
Fig. 9. A very clear view of *Tintinnopsis* showing the general structure. The tentaculoids, accessory membranelles, ciliary membrane, oral membranes, and column lobe are shown distinctly. The macronuclei are beginning to become oriented about the forming cytosome in preparation for division.











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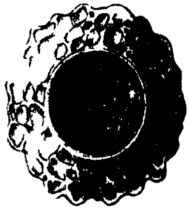

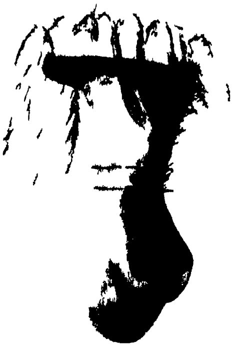

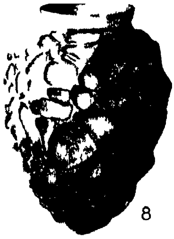

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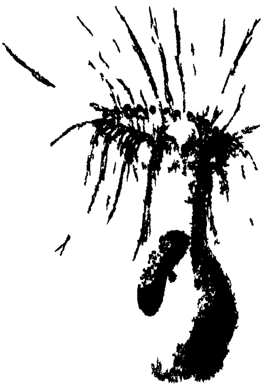


PLATE 13

Fig. 10. Showing an animal rather early in division. This specimen is heavily parasitized with *Karyoclastis tintinni* gen. nov., sp. nov., in a characteristic manner.

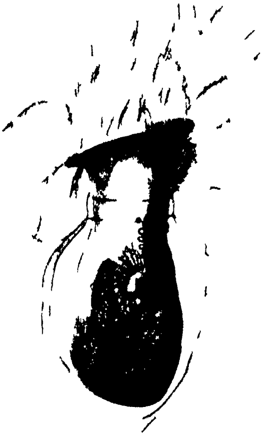
Fig. 11. *Karyoclastis tintinni* gen. nov., sp. nov., showing (*a*) an interkinetic individual, (*b*) one undergoing binary fission, and (*c*) one in multiple fission. Note the gray-staining mantle, clear central area, and central granule within which undergoes division. × 2000.

Fig. 12. Cytoplasmic phases of *Karyoclastis*. The parasites are shown escaping from the macronucleus. Some parasites are still within the wall while the greater number have already passed out into the cytoplasm.

Fig. 13. The earliest stage in division. The line of basal granules is shown beneath and at right angles to the ciliary membrane, cutting across the myonemes.

Fig. 14. Early fission stage. The new peristome is already formed and the nuclei have swung into position. The behavior of parts is indicated.

Fig. 15. Rather the same stage as figure 14 but oriented differently to show structures not indicated in that figure. The macronuclei have begun to constrict.



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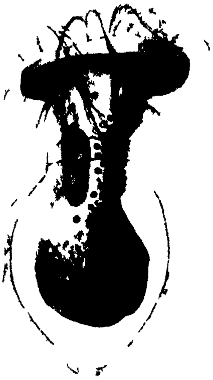

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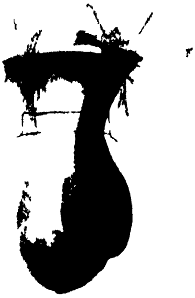

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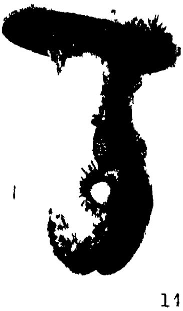

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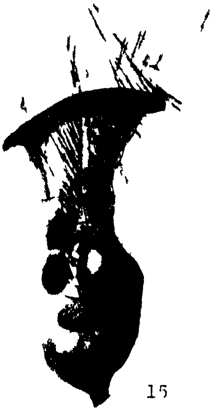

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PLATE 14

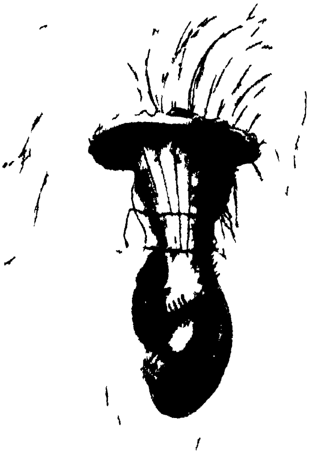
Fig. 16. Later than figure 15. The newly formed peristome has become independent. The micronuclei have divided and the macronuclei are not yet separated. The cytopyge is shown at one side.

Fig. 17. Division stage about the same time as figure 16 but showing the new membranelles growing out. The ring and motorium are distinct.

Fig. 18. A somewhat oblique view of a stage comparable to figures 15 and 16. The nuclei are in division.

Fig. 19. This specimen is somewhat later than previous figures. The nuclei somewhat obscure internal conditions. The motorium and ring are distinct. The tentaculoids are stained very pale but are fully expanded.

Fig. 20. A late fission stage showing the beginnings of separation of the two zooids. One nucleus has not yet divided, the others have separated and are passing into the two daughter individuals. The motorium is distinct.



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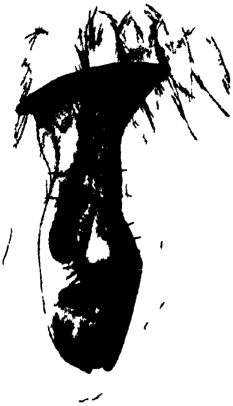

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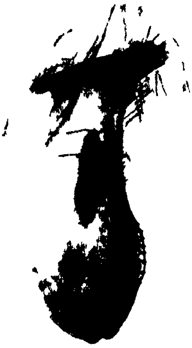

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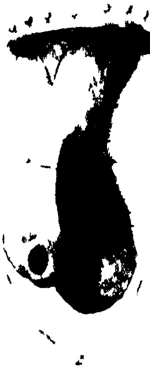

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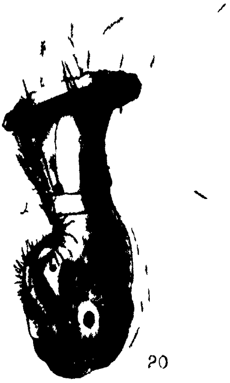

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PLATE 15

Fig. 21. A very late fission stage before the separation of the two daughters. The posterior animal is behind and will retain the lorica. All organ systems are formed and the animals are independent in every way except in the posterior connection.

Fig. 22. A single macronucleus and micronucleus especially showing the large trophic chromosomes. × 2000.

Fig. 23. Showing the polar massing of the trophic chromosomes in the macronucleus during division. The reconstruction band is clear. × 2000.

Fig. 24. A later stage of the fission of the macronucleus. Note the strand-like organization within the nucleus and the massing of the trophic chromosomes. × 2000.

Fig. 25. Daughter animals almost ready to separate.

Fig. 26. Degeneration form with two macronuclei. Note that the organs are lost.

Fig. 27. Micronucleus in telophase, the chromatin is becoming granular. × 2000.

Fig. 28. *Tintinnopsis* with stored faeces, some of which are being passed out by the ciliary membrane and attached to the lorica by the lateral column lobe. This animal is about to divide.

Fig. 29. Micronucleus in early telophase. The chromosomes are massed about the poles but have not yet lost their identity. × 2000.

Fig. 30. Abnormal form with a single macronucleus.

Fig. 31. A *Tintinnopsis* somewhat like figure 28. Not dividing but showing the path of faecal masses from the fundus of the lorica to the outside.

Fig. 32. A degenerate form free from a lorica, with but a single parasitized macronucleus.

Fig. 33. Interkinetic micronucleus. Note the granular structure. × 2000.

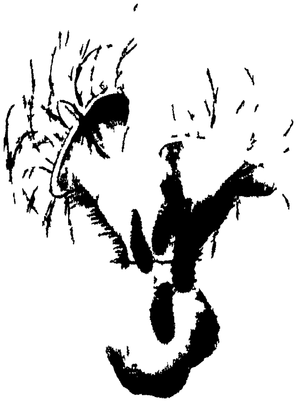
Fig. 34. Prophase micronucleus, enlarged, with the granules massed toward the center. × 2000.

Fig. 35. Prophase micronucleus. The chromosomes have formed. Note the polar caps. × 2000.

Fig. 36. Metaphase micronucleus. × 2000.

Fig. 37. Anaphase. The chromosomes are massed about the polar caps. × 2000.

Fig. 38. *Tintinnopsis* with faecal masses stored in the fundus of the lorica.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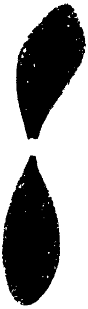
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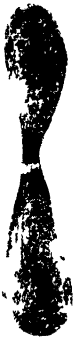
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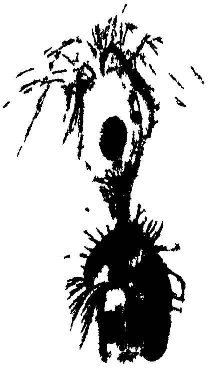
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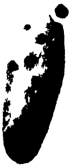
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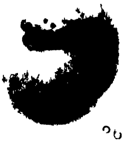
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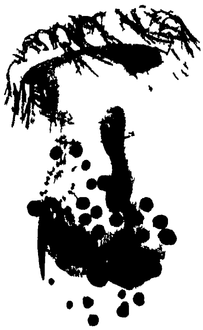

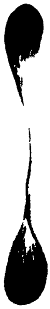
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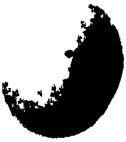
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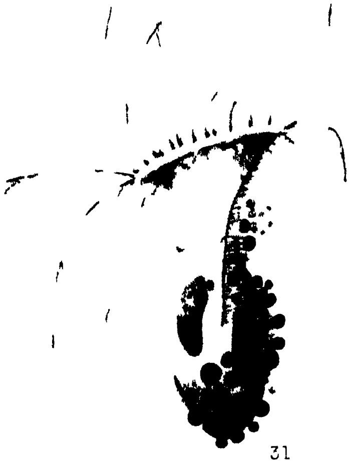
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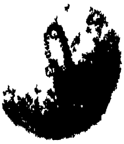
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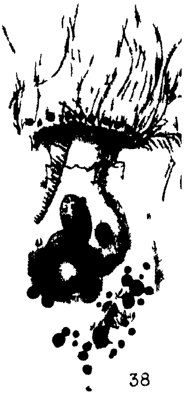
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ON TINTINNUS NERITICUS SP. NOV. FROM SAN FRANCISCO BAY

BY

ARTHUR SHACKLETON CAMPBELL

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BY

ARTHUR SHACKLETON CAMPBELL

In the abundant plankton found in the waters of San Francisco Bay there are numerous Tintinnoina. Among the various cosmopolitan species I found one, in some numbers, which is new. This I describe as *Tintinnus neriticus* sp. nov.

Tintinnus neriticus sp. nov.

Diagnosis.—*Tintinnus*-like lorica, stout and tubular, 2.47–3.15 oral diameters in length. Wall thick, with irregular flecks in a spiral on outer surface. Length, 380–470 microns. San Francisco Bay.

Description.—Lorica a stout tube (fig. 1) 2.47–3.15 (average 2.86) oral diameters in length. Oral diameter 0.97–1.40 aboral diameters. Oral and aboral margins irregular, not flaring, everted, or provided with a rim. Shaft a straight cylinder, contour of which is broken only by the spirally arranged irregular flecks on its surface.

The wall is homogeneous when examined at a magnification of 225 diameters, but when examined at 1050 diameters under optimum conditions, it may be resolved into alveoles, arranged in three or four layers. The irregular flecks on the outer surface of the wall show three or four layers of alveoles of a denser, more refractile nature than the wall matrix. The wall is 4.2 microns or 0.04 oral diameters across and is uniform except for the surface flecks. The general regular contour of the lorica is broken only by these patches, which are arranged along a slight ridge in a leiotropic spiral. There are ten or twelve of these spirals determinable in favorable individuals. Some individuals show little or no trace of the spiral organization and may be forms in which conglutination has obliterated all traces of structure.

The lumen of the lorica is cylindrical or barely tapering aborally and smooth surfaced, following the contour closely.

I have seen the animals on several occasions alive. One preserved individual (fig. 1) had two irregular macronuclei, each 20 by 40 microns, and two spherical micronuclei, each 4 microns in diameter. There are about 20 membranelles, each one of which is 35 microns in length. The cytosome is trumpet-like, 190 microns in length, and 70 microns across the frontal field, with a constricted throat below the level of the frontal field. A peduncle attaches the animal posteriorly to one face of the lumen.

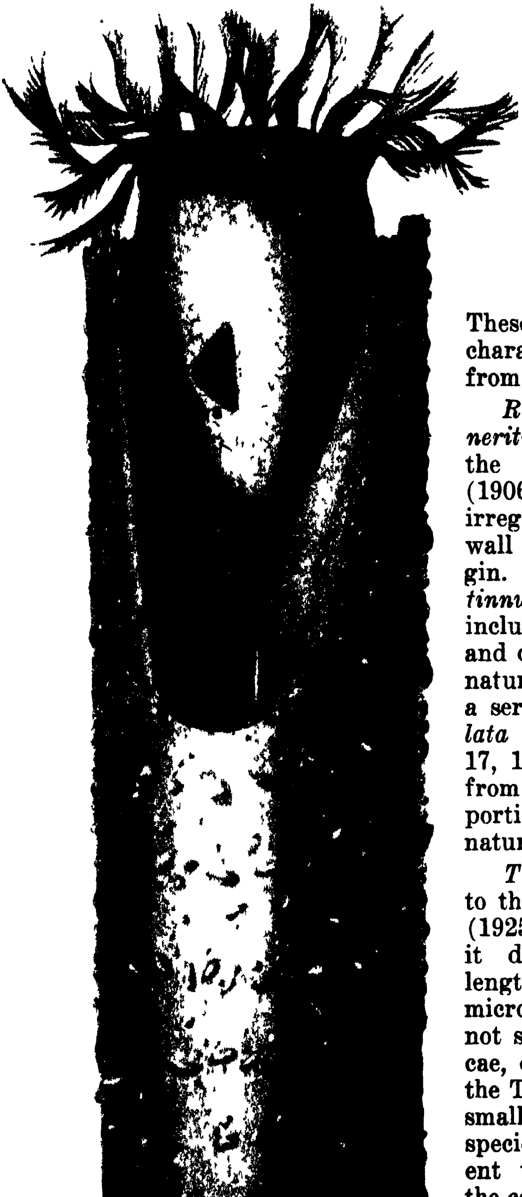

Fig. 1. *Tintinnus neriticus* sp. nov., from San Francisco Bay, showing the preserved animal. × 280.

Measurements of five loricae selected at random from my material are given in the table below:

Length	Oral diameter	Mid-way	Aboral diameter
470	150	150	150
465	175	165	172
450	145	143	140
380	160	155	148
380	120	95	90

These measurements are very characteristic of a large series from this station.

Relationships.—*Tintinnus neriticus* is related in form to the *fraknoii*-series of Brandt (1906, 1907), but it differs in the irregular character of its thick wall and in the irregular margin. It belongs in the genus *Tintinnus* if the latter genus is to include forms with tubular body and open aborally, whatever the nature of the wall. It may form a series with *T. fraknoii maculata* (Brandt, 1906, pl. 65, figs. 17, 17*a*, 18, 18*a*; 1907, p. 424) from which it differs in its proportions and especially in the nature of its thicker wall.

Tintinnus neriticus is similar to the form described by Wulff (1925) from the North Sea, but it differs in its dimensions, length 150, oral diameter 35 microns. Wulff (1925), who has not seen the animal of his loricae, doubtfully places it among the Tintinnoina. This may be a smaller form of the present species, occurring under different temperature conditions in the cold waters of the North Sea.

Comparisons.—This distinctive form is recognizable by its thick, soft wall and may be separated from *Tintinnus fraknoii maculatus* Brandt (1907) by its proportions and by the character of the wall which, in the latter species, is thin and homogeneous. It differs from *T. serratus* Kofoid not only in the above-noted features, but also in lacking regular teeth on the oral margin.

Occurrence.—Found in San Francisco Bay at the Key Route Mole in March–May. Frequently the most abundant species found. Since there is a paucity of living animals and an abundance of empty loricae in our net catches, the animals may either desert their houses freely when caught in the net, or may have left them previously because of local adverse conditions created by sewage and industrial wastes.

Discussion

The spiral arrangement of the irregular patches on the surface of the lorica is of great interest. These patches are a morphological evidence of the manner of construction of the lorica which is a product of the cytosome, its matrix arising out of the body by secretion and moulded by the neuromotor organs into a characteristic tube. This arrangement of substances is carried on at first in the oral region and is added to as the animal elongates in separation, building the lorica as it progresses and distributing the lorical substances spirally by rotation about the long axis. The spiral may be a device to increase surface and assist in flotation.

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AN EXPERIMENTAL STUDY OF THE EFFECTS OF DYES, OF DYE MIXTURES, AND OF DISINFECTANTS UPON ENDAMOEBA GINGIVALIS (GROS) IN VITRO

BY

DOROTHY ANN KOCH

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INTRODUCTION AND ACKNOWLEDGMENTS

The fact that Boeck and Drbohlav (1925) succeeded in growing parasitic amoebae in artificial media added a great stimulus to the interest in this field of medical zoology. Howitt (1925*a*, *b*) and Drbohlav (1925*b*) have had success in the cultivation of *Endamoeba gingivalis* (Gros), the amoeba of the mouth, and Howitt (1925*a*, *b*), particularly, has observed the effect of a variety of physical and chemical agents upon this amoeba *in vitro*. However, until such cultures are freed of bacteria and other contaminating organisms, the work of determining the rôle of the amoeba in diseases of the mouth and gum tissue will be much impeded. In the hope, therefore, that by means of certain dyes and dye mixtures the bacteria might be eliminated, or cut down to the point where other means of isolation of the amoeba would be successful, the present experimental work was undertaken.

Since Churchman (1912) first started his work on the bactericidal action of dyes, much has been done and a number of theories as to the action of dyestuffs upon bacteria have been set forth. Outstanding among these workers have been Churchman (1912*a*, *b*, 1920, 1921–22, 1923, 1925), Browning and Gilmour (1913), Browning and Gulbransen (1919), Browning, Gulbransen, and Kenneway (1919), Kligler (1918), Norton and Davis (1923), Stearn and Stearn (1923, 1924*a*, *b*, *c*), Loeb (1922) and Felton and Dougherty (1922*b*). Gentian violet was the dye chiefly used for the earlier experimental work and the action of this was greatly altered by the amount and nature of the protein present (Bechold, 1909); Kendall and Bly, 1922; Robertson, 1908; Kligler, 1918; Churchman, 1921). It is not proposed here to enter into a theoretical consideration of the action of dyes; the present work is concerned only with the mass action upon the proteins present and whether or not a selective action between the bacterial protein and the protozoan protein is exercised by any of the dyes or dye combinations used. However, because of the mode of cultivation of the organisms dealt with, a considerable number of factors influencing inhibition come into play, and the results may perhaps be a little more easily understood if a brief summary of some of the more recent theories of the action of dyestuffs is given.

Ehrlich introduced the name "chemotherapy" for the investigation of substances acting specifically on pathogenic infections, that is, substances being maximally parisitropic and minimally organotropic. The fact that, in many cases, a remedy acting against a certain infection *in vitro* is without action on the parasites *in vivo* is not yet clearly understood. At the present time experimental results are mainly interpreted in terms of direct parasiticidal action. Loeb (1922) advanced the theory that "a protein is an amphoteric substance which combines with either acids or bases, according to whether it is a solution whose pH is respectively either less or greater than a certain value known as the isoelectric point of the protein in question." He demonstrated that the pH regulated the amount of dye taken up by the bacterial protein and that a point was reached where the bacterial protein no longer took up the basic dye, but, as the acidity was increased, combined more and more readily with the acid dye. Stearn and Stearn (1924*c*) show that the maximum bacteriostatic effect is produced by the formation of this "dye-protein compound." According to this theory, a protein is easily liberated from its combination, so that, unless some other agent is active in actually destroying the bacterial protein while growth is inhibited by the dye, the organism is merely awaiting an opportunity to develop. This same effect will be shown to apply to the amoebae here studied. Irwin (1925) also considers this action to be a chemical combination of the dye with a constituent of the cell; a "reversible pseudomolecular reaction."

Recently Stearn and Stearn (1926) have shown that the degree of chemical combination of the dye with the protein is directly dependent upon the pH of the medium in which the experiment is carried on. They insist that a buffered medium is the only reliable agent for experimental use. The following experiments were all carried on with a medium having an initial pH of 7.6. At the present time a buffered solution for the growth of amoebae is being perfected by us.

The composition of the medium, the mass effect of the bacteria, and the reaction of the medium must all be taken into consideration. Since the cultures used for the present work were grown in a liquid medium composed of Locke's solution and egg albumin over coagulated whole egg, and since they contained in addition to the amoebae a mass of bacteria, chiefly streptococci and staphylococci, it can readily be seen what a variety of factors were present to influence the action of the dyestuffs used.

Opposing the chemical theory of the action of dyestuffs upon proteins is the physical theory, which accounts for the action by the principle of absorption. This theory is not widely accepted at the present time, but it is probable that both physical and chemical phenomena enter into the mechanism of dye therapy.

Browning and Gulbransen (1918), Hewlitt (1917), Dakin and Dunham (1918), Gay and Beckwith (1922), and others have found that the flavine and acridine compounds are most active against bacteria in protein solutions. The dyes of this group and the phenylmethane dyes have therefore been the chief ones used in this study.

With the exception of the benzopurpurin series of dyes developed by Ehrlich and Shiga and Bayer "205," a substance similar to the afridol violet developed by Mesnil and Nicolle, dyes have not been extensively used or investigated in their relation to Protozoa. The substances mentioned were toxic for trypanosomes. Robertson (1925) has studied the influence of dyes upon Infusoria (*Enchelys*) and

Fairbrother and Renshaw (1922) have used *Paramecium* in an investigation upon the relation between chemical constitution and antiseptic action. The latter have also studied the effect of certain dyes upon the Protozoa of the mouth.

Since the object of the present work was not only to study the effect of dyes upon *Endamoeba gingivalis*, but also to attempt to get a selective action which would eliminate from the cultures a majority of the accompanying bacteria, and since these cultures were in no way comparable to those used in the above-named studies, the results will be seen to vary in some respects from those of previous workers.

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MATERIAL AND METHODS

Two strains of *Endamoeba gingivalis* were used for the present work. The strain obtained from Howitt, No. 38, came from a deep gingival pocket. It has been under artificial cultivation since February 26, 1925, a period of 18 months, and has undergone 250 transplants. The other strain used, No. 56, came from a case of slight gingivitis. It has undergone 24 transplants to date. The amoebae were grown in a modification of Boeck and Drbohlav's medium as described by Howitt (1926*a*), being essentially a solid base of coagulated egg to which was added a modified Locke's solution containing egg albumin. This medium was also employed in the experimental work to be described.

Stock solutions of the dyes to be used were made just before an experiment was performed, so that no solution was more than a day or two old. It is proposed by the Commission on the Standardization of Biological Stains (1925) that, in order to bring about more uniform results, saturated solutions be used as stock solutions. Since this work was started, however, they report that even saturated solutions vary, as the inert material in the dry stain often prevents complete solubility. It was felt that since the work here given was started with solutions made from a weighed quantity of dry stain, this method should be continued in order to secure comparable results. In all cases, the same make of stain was used throughout and the distributor's name is given. All German dyes used were bought before the war. Glassware was rinsed in 1 per cent hydrochloric acid.

Whenever possible, one gram of the material to be used was accurately weighed and placed in a small sterile flask. To this 100 cc. of sterile distilled water were added, the stopper was covered with paper or a rubber cap, and the whole sterilized in the autoclave for 20 minutes at 15 pounds. In the case of proflavine, the solubility of the dye did not permit a stock solution of 1–100.

TABLE 1

METHOD OF MAKING DYE DILUTIONS

Dye in liquid media		Liquid media	Final dilution
Dilution	Amount		
	1 gm	10 cc (H_2O)	1-100
1-100	1 cc	9 cc	1 1,000
1-1,000	5 cc	2 5 cc	1-1,500
1-1,000	5 cc	5 cc	1-2,000
1-1,000	3 cc.	4 5 cc	1-2,500
1-1,000	3 cc	6 cc	1-3,000
1-1,000	1 cc	9 cc	1-10,000
1 10,000	5 cc	2 5 cc	1-15,000
1 10,000	5 cc	5 cc	1 20,000

Dilutions were made by adding the stock dye to the filtered Locke's and albumin, carrying over from one tube to the next until dilutions of 1–1,000, 1–10,000, and 1–100,000, had been made, using the same technique as is employed for making dilutions for an agglutination test. From these three dilutions a graded series was prepared by adding varying amounts of the dye to varying amounts of the liquid media; that is, a definite amount of media was added to a tube containing the coagulated egg and to this was added a measured amount of one of the above mentioned concentrations of dye. A portion of the table used in making the dilutions is given to show the procedure followed.

In adding dye to media, the lowest dilutions were always made first so as not to carry over any excess dye. It will be seen that the total liquid media above the culture varied from 7.5 to 11 cc. This variation was not enough to influence growth of amoebae. After a series of tubes had been filled they were warmed to 37° F. and inoculated.

At first, transplants were made by inoculating three tubes—two into dye dilution and one into Locke's and albumin as a control—from every 48-hour stock tube. However, the stock tubes varied so much in the number of amoebae present that, in order to get results that could be read without examining a great number of controls, a system whereby only one control for every ten tubes was needed was devised. This was so much more satisfactory than the first method that all previous experiments were repeated. The results here given were therefore all made with the same method of inoculation, which was as follows: About one cubic centimeter of growth from the bottom of each stock tube was removed and placed in a warmed test tube or small flask, which was left in a 37° water bath. This liquid, with many amoebae, was then drawn up into a 10 cc. pipette having a wide mouth, and each experimental tube inocluated with 0.5 cc. of the culture. The pH of the medium was 7.4–7.6. If a few precautions are taken, the results will be very satisfactory. The culture must be kept warm at all times and as free as possible from lumps of media. The amoebae stick to these solid surfaces and, by inoculating with them, one tube is liable to receive a great many more organisms than the next. Also, the flask of culture must be gently but thoroughly shaken before some of it is drawn up into the pipette and the work must be rapid, as the amoebae are heavy and tend to sink to the bottom. With everything at hand one row of tubes, nine experimental and one control, can be inoculated in about one minute. Careful checks have shown that this proportion of control tubes is satisfactory.

As soon as inoculated, the tubes were placed in the incubator at 37° and readings were taken at the end of 24, 48, and 72 hours. By counting the number of amoebae, an estimate of the increase or inhibition of growth above or below normal is possible. Howitt (1925) devised a simple method for counting the number of amoebae present in a coverslip preparation, and it was thought wise as well as convenient to follow this procedure in order that the results of our experiments on *Endamoeba gingivalis* might be satisfactorily compared with hers. Normal or control tubes contained 10 cc. of Locke albumin over a slant of coagulated egg, i.e., the ordinary culture medium mentioned above and used by us for stock cultures. Growth from the bottom of a culture tube was therefore removed with a sterile pipette and a definite number of drops placed on a slide and covered with a 22 × 22 coverglass. The preparation was placed on a warm stage and the number of amoebae counted in a definite number of fields—about 130. This allowed eight rows from left to right. In counting, 6.5× oculars and the 16 mm. objective were used, giving a magnification of 240 diameters. The control tubes were designated by letters, and the letter following a number refers to the corresponding control tube.

The dilutions used ranged from 1–1000 to 1–1,000,000, twenty-eight in all for each dye. By running such a series for each dye used, the end point for the amoebicidal action could be determined and the experiment repeated over a finer range if there was a promise of bacteriostatic action as well. Table 2 shows the range of dilutions used and the method of recording results. Controls are at the extreme right and in the horizontal column of results to which they correspond.

EXPERIMENTAL

The dyes used are grouped below according to the chromospores present, the classification being that of the Commission on the Standardization of Biological Stains (1925).

- Quinone-imide group.
  - Oxazines: *Nile blue sulfate, Nile blue chloride, Naphthol blue.*
  - Azines: *Phenosafranin.*
- Phenyl-methane dyes.
  - Diphenyl-methanes: *Auramin.*
  - Diamino tri-phenyl methanes: *Malachite green oxylate, Brilliant green, New solid green 3B.*
  - Triamino tri-phenyl methanes: *Basic fuchsin, Acid fuchsin, Crystal violet, Ethyl violet, Acid violet.*
- Acridine group: *Acridine yellow R, Acriflavine, Proflavine, Acridine orange.*

1. QUINONE-IMIDE GROUP

OXAZINES

The dyes of this group are of interest because of their metachromatic properties and because of their affinity for fats. Those studied were all basic dyes. Apparently *E. gingivalis* ingests fat from the egg yolk of the medium, and this can often be seen within the motile organism in the form of minute refractile granules (Howitt, 1925).

Nile blue sulfate (*Nile blue A*, Grübler).—This dye is only weakly amoebicidal. It has very little effect upon the bacterial flora accompanying the amoeba in the cultures, but affords an excellent opportunity for the observation of amoeboid movement. The lipoidal globules within the cell take up a delicate blue stain, so that the endoplasm and ectoplasm are clearly defined. At the end of 72 hours the amoebae were definitely colored in dilutions ranging from 1–30,000 to 1–50,000, and, except for the stain, were in all ways active and normal in appearance. Pseudopodia were hyaline and the outer part of the endoplasm (that nearest the cell wall) containing no food material, also remained clear, as did the bacteria surrounding the organism. As Nile blue sulfate is not fat-soluble, but combines readily with fatty acids, it would seem that the ingested material within the cell was in this form. The lowest dilution in which the amoebae lived in this dye was 1–30,000, showing it to be one of the least amoebicidal dyes used. Bacteria were not appreciably inhibited. Becker has found that Nile blue becomes reduced to the leuco-base in an oxygen-free medium. Reduction was apparent in the butt of the tube where partial anaerobic conditions prevail. Amoebae living in this dicolorized dye were stained blue, however.

Nile blue chlorhydrate (*Nile blue 2B*, Grübler).—As has been mentioned, in all cases dyes were made up as follows: One gram, accurately weighed, was dissolved in 100 cc. of sterile distilled water. This stock solution was sterilized. Dilutions of 1–1,000, 1–10,000, and 1–100,000 were made up from the stock. These last dilutions were made in sterile, filtered Locke's albumin. In the case of this dye, the 1–1,000 and 1–10,000 dilutions precipitated immediately, the dye changing from blue to a deep magenta. The 1–100,000 solution, made from the 1–10,000, remained blue and unprecipitated. Therefore all dilutions stronger than 1–200,000 were of precipitated dye. Apparently the change was due to the chemical constitution of the Locke's. The media again changed to a blue when 0.5 cc. of the culture at a pH of 6.8 was added. Results with this dye were similar to those for Nile blue sulfate, but they are not considered accurate because of the precipitation.

Naphthol blue (*Meldola's blue*) (Coleman and Bell).—As in the case of Nile blue sulfate, this dye stained active amoebae without killing them. It was tolerated by *E. gingivalis* in a 1–70,000 dilution and was not noticeably bacteriostatic. In the lower dilutions, where the dye was visible, it became discolored and brown at the bottom of the tube. When this lower part was removed with a pipette and placed on a slide, an oxidizing process apparently ensued, as the material turned blue-green almost at once in the presence of air. It is assumed that the dye had undergone reduction, but whether this was due to bacteria or Protozoa, or both, is uncertain. Amoebae examined from the discolored sediment were definitely stained blue while still motile and active.

The *oxazine dyes* therefore are only moderately amoebicidal *in vitro* and are not bacteriostatic in dilutions which permit the growth of *E. gingivalis.* This is of interest because Fairbrother and Renshaw (1922) found the group to be among the most active toward *Paramecium.* They also found the oxazines to be bactericidal to gram-positive organisms in dilutions tolerated by *Paramecium.*

AZINES

Phenosafranin (Coleman and Bell).—This strongly basic dye was the only one studied in this group. While it was very weakly amoebicidal and there was no apparent inhibition of bacteria in a 1–6,000 dilution in 24 hours, at the end of 72 hours there was practically no disintegration at the bottom of the tube and the rod forms seemed to have disappeared from the cultures. The cocci were not affected, there being an abundant growth of *Staphylococcus.* The amoebae were large and very active, but normal in appearance. Amoebae in the control tubes were smaller. This was taken to indicate not cessation of division, but evidence that conditions for development were favorable.

Because of the inhibition of putrefaction of the egg slant, and the good growth of amoebae in this dye, it was later used with some success in combination with other dyes.

PHENYL-METHANE DYES

Until recently, this group has been the most widely used for experimental bacteriostasis. Gentian violet, brilliant green, malachite green, and the fuchsins have all been used for differentiation because of their selective action. It was therefore hoped that with the first three named some attack might be made upon the *Staphylococcus* present. Gay and Morrison (1921) found new fast green 3B and basic fuchsin bactericidal for *Staphylococcus* and *Streptococcus* in a final dilution of 1–2,000. Churchman (1921–22) found acid fuchsin bactericidal to the "commoner gram-negative bacteria" and that it inhibited sporulation of *B. subtilis* (1925). Fairbrother and Renshaw (1922) found ethyl violet, malachite green oxylate, and auramine O strongly active against *Paramecium.*

DIPHENYL-METHANES

Auramin (Grübler).—It was hoped to use auramine O in this experiment, but the material which was procured was so impure and insoluble that auramin was substituted. This dye was soluble in a 1–100 solution only when hot. The dye was therefore dissolved and further dilutions made at once from the hot solution. No change appeared to take place upon heating. The dye was lethal to *E. gingivalis* in a 1–60,000 dilution in 72 hours, making it only moderately amoebicidal. Bacterial growth was heavy in the 1–10,000 tube. The dye did not discolor upon incubation.

DIAMINO-TRIPHENYL METHANES

Malachite green oxytate (Coleman and Bell).—The lethal dilution of this dye for *E. gingivalis* was 1–20,000. Fairbrother and Renshaw (1922) found it to be very active against *Paramecium* and also a bactericidal agent, and ranked it with auramine O and ethyl violet. In the presence of the albumin in the medium, however, malachite green oxylate proved to be of little use in the inhibition of either bacteria or Protozoa in our tests.

New solid green 3B (Coleman and Bell).—This dye was only weakly amoebicidal, slight growth taking place in a 1–10,000 solution after 48 and 72 hours. At the end of 24 hours the living amoebae in the 1–20,000 and 1–30,000 dilutions were faintly colored. After 48 hours' incubation, except for the 1–7,000 dilution, the dye in the experimental tubes had faded almost completely. Bacteria were inhibited in dilutions as weak as 1–200,000. Gay and Morrison (1921) found it strongly active against *Staphylococcus* and *Streptococcus.*

Brilliant green (Grübler).—The use of this dye for inhibiting the colon bacillus in stools lead to its application here. It has, apparently, exactly the same amoebicidal powers as new solid green 3B. No inhibition of bacteria was noticed. Banting (1924) has found a 1 per cent solution to be germicidal without being harmful to tissues and it has been used in this connection for oral disinfection.

TRIAMINO-TRIPHENYL METHANES

Basic fuchsin (Grübler).—Basic fuchsin was found by Gay and Morrison (1921) bactericidal for both *Staphylococcus* and *Streptococcus* in a dilution of 1–2,000. These two organisms are constantly recovered from the mouth in great numbers when cultures are made for amoebae. It was found that this dye was lethal to *E. gingivalis in vitro* in the same dilution (1–9,000) as the last two named phenylmethanes. This dilution, however, was too great to inhibit bacterial growth.

Acid fuchsin (Grübler).—This dye is a sulfonated derivative of basic fuchsin and is generally a mixture of several compounds. It was the most weakly amoebicidal dye studied, reproduction of amoebae taking place in a 1–200 dilution as rapidly as in the corresponding control tube. Inhibition of bacteria between the dilutions of 1–600 and 1–200 was sufficient to prolong the life of the culture to 10 days without transplants or change of media. The last two dilutions (1–200 and 1–400) were made from a 1–100 dilution of dye in Locke's solution, so as not to add too great a volume of water to the culture media. The dye completely penetrated the coagulated egg slant. In the light of the results of other workers, we may therefore conclude that the action of the dye on living protoplasm was almost at a minimum in this instance.

Ethyl violet (Grübler).—The lethal dilution of ethyl violet for *E. gingivalis* was 1–10,000. It therefore corresponds very closely in its action to the other members of its group. At the end of 24 hours, the spiral organisms often present in cultures had disappeared and no motility of the bacteria was seen.

Acid violet (Grübler).—Although this dye is supposed to be lethal to *Staphylococcus* in dilutions of 1–20,000, in our work it had little effect. Bacteria were not noticeably inhibited and its amoebicidal properties were poor, growth being obtained in a dilution of 1–1,000.

It may be said, from the above experiments, that the *phenylmethane* dyes vary greatly in their amoebicidal properties, but that, within the group, of the dyes studied the diphenyl-methanes are the most active, the triamino-triphenyl methanes are the next, and the diamino-triphenyl methanes are the least active against *Endamoeba gingivalis in vitro.* In some cases bacteria are inhibited in dilutions permitting the growth of amoebae but such inhibition is not great enough to elminate bacteria from the cultures even if successive transfers are made.

These dyes are used in bacteriological work particularly because of their selective action, brilliant green inhibiting the colon bacillus, acid fuchsin inhibiting sporulation of *B. subtilis*, new solid green 3*B* inhibiting *Staphylococcus* and *Streptococcus;* and some are used for their bactericidal properties. No such lethal action could be obtained in the present work because the nature of the medium inhibited the action of the dyes and the dilutions which killed bacteria were also lethal for *E. gingivalis.*

Table 2 gives the systematic arrangement of the dyes used and the lethal dilution of each dye for *E. gingivalis.* Full results for each dilution are shown in table 3.

TABLE 2

SYSTEMATIC RELATION OF DYES AND HIGHEST LETHAL DILUTION FOR EACH DYE USED

Dye	Group	Derivative	Highest lethal dilution for *E. gingivalis* in 72 hours
Acid fuchsin	Phenyl-methane	Triamino-triphenyl methane	Below 1-200
Phenosafranin	Quinone-imide	Azine	
Acid violet	Phenyl-methane	Triamino-triphenyl methane	Below 1-1,000
New solid green 3B	Phenyl-methane	Diamino-diphenyl methane	1-9,000
Brilliant green	Phenyl-methane	Diamino-diphenyl methane	1-9,000
Basic fuchsin	Phenyl-methane	Triamino-triphenyl methane	1-9,000
Malachite green oxylate	Phenyl-methane	Diamino-triphenyl methane	1-20,000
Ethyl violet	Phenyl-methane	Triamino-triphenyl methane	1-20,000
Nile blue A	Quinone-imide	Oxazine	1-20,000
Naphthol blue	Quinone-imide	Oxazine	1-60,000
Auramine	Phenyl-methane	Diphenyl-methane	1-60,000
Acridine orange	Acridine	Acridine	1-60,000
Acridine yellow R	Acridine	Acridine	1-90,000
Acriflavine	Acridine	Acridine	1-200,000

ACRIDINE GROUP

The acridine dyes are the most powerfully bactericidal dyes in present use. As shown by Browning, Gulbransen, and Kenneway (1919), Hewlitt (1917), Dakin and Dunham (1918), and later workers, the flavine and acridine compounds are most active against bacteria in protein solution. They would therefore be of particular value in a medium containing egg albumin.

Proflavine (National Aniline Co.).—This dye gave very unsatisfactory results as it was highly insoluble, some of the dye remaining undissolved in a 1–500 solution. A saturated solution was therefore used, dilutions being made from that, but unfortunately results cannot be compared with those of other dyes used in this work. The lethal dilution of a saturated solution was 1–200,000 for *E. gingivalis.*

Acridine yellow R (National Aniline Co.).—The amoebicidal action of this dye was a 1–90,000 dilution at the end of 48 hours. The inhibition of bacteria was slight, good growth showing in the 1–50,000 dilution. It is interesting to note that the spiral organisms present in the culture were more resistant to the dye than were the bacteria.

Acriflavine (National Aniline Co.).—Browning and his coworkers (1917, 1918) first found this substance to have a powerful antibacterial action when augmented by the presence of serum. In the experiments here described this dye was the most powerful amoebicidal agent used, being lethal in a dilution of 1–100,000 in 24 hours and in a dilution of 1–200,000 in 48 hours. It will be seen from table 3 that all the acridine compounds studied were slow in their action, the maximum effect being produced in 72 hours. At the end of 48 hours bacteria were noticeably inhibited in a dilution of 1–200,000. This inhibition had dropped to the 1–100,000 tube at the end of 72 hours. Upon transplanting to normal media, bacterial growth was as heavy as in the stock cultures, showing the action of the dye to be merely inhibitory; a prolonged cultivation in the dye solution killed the amoebae.

It may therefore be said that while acriflavine is a powerful amoebicidal dye *in vitro,* it is not so favorable for the prolongation of life when used in a culture as is a dye having immediate action but little effect upon the amoebae, its chief value lying in the inhibition of bacteria.

IODATES

At the suggestion of Dr. C. B. Lipman, sodium and potassium iodate were used in an attempt to eliminate bacteria. He has found these substances to be very effective in the destruction of certain bacteria of the soil. Dilutions used in this work were made as in the case of the dye experiments and the work carried on in the way described above. While the drugs were not strongly amoebicidal, bacterial multiplication was so lessened that cultures were kept alive for as long as 10 days without transplanting, the usual disintegration of the egg in the butt of the tube being checked. At the end of this time there was good growth in dilutions ranging from 1–6,000 to 1–9,500, although the amoebae were very small in the stronger concentrations. Transplants, however, at the end of 12 days failed to show growth of amoebae. The pH of the medium had dropped less than in the controls, so that it would seem that the destructive action of the bacteria is due, not so much to their effect upon the pH as to other products of metabolism which may have a toxic effect upon amoebae.

Potassium iodate proved to be similar to the sodium compound in its effect, both being lethal in a 1–6,000 dilution in 72 hours. The iodates are slow in their action and are comparable to acriflavine in this respect.

Cocci appeared to be unaffected by either iodate while other bacteria were cut down to the point where there appeared at times to be almost a pure culture of cocci and amoebae. The results of the experiments described above are shown in table 4.

OTHER CHEMICALS

Wilkes-Weiss and Weiss (1926) have recently reported successful purification of cultures of *Spirochaeta pallida* by chemical means. They added *B. coli* and *Staphylococcus* to pure cultures of the spirochaetes. These mixtures were placed in a strong solution of the disinfectant in either physiological salt solution or distilled water and exposed for short periods of time. In this way a rapid bactericidal action was obtained and the spirochaetes removed before they had been killed. This "intrinsic" method has the advantage of allowing the chemical to act in a medium free from protein, and will, according to the authors, kill both types of organisms added as contaminants, with tricresol 1–100 in 1 minute, tetrachloracetic acid 1–100 in 15 minutes, or formaldehyde 1–20 in 5 minutes.

TABLE 4

EFFECT OF SODIUM IODATE ON ENDAMOEBA GINGIVALIS AND THE BACTERIA ACCOMPANYING IT IN CULTURE

Dilution	Amoeba count				Final pH	Remarks
	24 hrs.	48 hrs.	72 hrs.	96 hrs.		
1- 1,000	0*d*	0*d*	0*d*			Bacteria dead
2,000	0*d*	0*d*	0*d*			
3,000	0*c*	1*c*	0*c*			
4,000	0*c*	1*c*	0*c*			
5,000	0*c*	6*c*	0*c*			
6,000	1*c*	3*c*	0*c*			
7,000	0*b*	4*b*	4*b*	4*b*		
8,000	4*b*	82*b*	392*b*	352*b*		
9,000	0*b*	112*b*	292*b*	224*b*		
10,000	17*a*	30*a*	44*a*	728*a*	6.2	Bacteria low
20,000	20*a*	816*a*	336*a*	600*a*	6.4	Bacteria low
30,000	49*a*	1536*a*	1336*a*	280*a*	6.4	Bacteria low
40,000	48*a*	1280*a*	1136*a*	280*a*	6.6	Butt of slant disintegrating
50,000	21*a*	672*a*	976*a*	224*a*	6.6	Butt of slant disintegrating
Control *a*	40	1256	1072	184	6.6	Butt disintegrating
Control *b*	8	264	280	56		Butt disintegrating
Control *c*	2	56	344			
Control *d*	11	216	216			

Letters refer to control tubes at bottom of page.

In attempting this method of purification, the drug was added to sterile Locke's solution and the amoebae placed in the solution for varying lengths of time and transferred to the regular culture medium. All bacteria were destroyed by the formaldehyde, while the tricresol seemed to have little effect on the contaminants. Amoebae were destroyed in all cases.

TABLE 5

EFFECT OF EXPOSING AMOEBAE FOR SHORT PERIODS OF TIME TO STRONG DISINFECTANTS

Chemical	Dilution	Exposure	Growth in 48 hours
Tricresol	1–100	5 min.	0
Tricresol	1–100	1 min.	0
Formaldehyde	1–20	1 min.	0
Formaldehyde	1–20	2 min.	0
Formaldehyde	1–20	5 min.	0

DYE MIXTURES

Churchman (1923) reports a great deal of success with mixtures of dyes, particularly acriflavine and gentian violet. Such a combination kills in a dilution where either alone will not, giving a "supplementary selective bacteriostasis." Equal quantities of the dyes were mixed and dissolved in water in a strength of 1–2,000—a solution "non-irritant for inflamed mucous membranes." Bell (1924) has used these findings as a basis for a great deal of clinical evidence that aniline dyes and mixtures of dyes are effective in the treatment of certain infections. He has successfully treated, among other cases, whooping cough, pneumonia, erysipelas, and *steptococcus* septicemia. Bell has used chiefly a mixture of acriflavine and gentian violet prepared by Churchman. He has also used the dyes separately.

In the experiments described below, there was only a very slight increase in the amoebicidal power of a mixture of dyes over either dye used alone, but in some instances bacteria were greatly inhibited, with marked results upon the longevity of the culture amoebae.

In preparing the dye mixtures, one gram each of the substances to be used was accurately weighed, both were placed in a small pyrex flask, 100 cc. of sterile distilled water added, and the mixture autoclaved to sterilize. Dilutions were made as for the single dyes, and results recorded in the same manner. Table 5 gives the results of the experiments on the following solutions:

Acriflavine and *Acridine orange.*
Acriflavine and *Sodium iodate.*
Acriflavine and *Gentian violet.*
Acriflavine and *Phenosafranin.*
Acriflavine and *Ethyl violet.*
Acriflavine, Phenosafranin, and *Gentian violet.*

It is interesting to note that, in the mixture of *phenosafranin* and *acriflavine,* at the end of 24 hours the control showed only numerous bacteria, no multiplication taking place in experimental tubes. This mixture of dyes was used because phenosafranin had been active among rod forms which contaminated the culture, and by means of this mixture these bacteria were entirely eliminated. When the 48-hour 1–100,000 dye culture was transplanted to sterile normal media, growth of bacteria occurred. To this tube, at the end of 48 hours, whole sterile guinea pig blood was added. The bacteriostatic power of the dye mixture was very marked. Red blood cells remained unhaemolized at the end of 72 hours, while in the tubes containing no dye, the bacteria multiplied so rapidly that in 24 hours all the blood cells were broken down.

The original dilution tubes were kept for 12 days, at the end of which time the cultures in the 1–300,000, 1–400,000, and 1–500,000 tubes were transplanted to fresh media containing the same dilution of dye. There was good growth of amoebae at the end of 24 hours' incubation, showing that the original culture was still alive after 12 days. Bacterial growth was very slight. Because the amoebae feed chiefly on bacteria *in vitro,* and because these were so greatly reduced, washed guinea pig red blood cells were added at the end of 24 hours. Ingestion of red cells by amoebae occurred in many cases. This confirms Howitt's (1925) results on the ingestion of blood by *E. gingivalis.* Drbohlav (1926) has been unable to observe this phenomenon.

At the present time, no culture has been kept alive for more than 12 days without transplanting or adding fresh media. Control tubes kept for over 5 days become putrid, the egg slant disintegrates, and amoebae can rarely be recovered after 6 days.

Gentian violet and *acriflavine* also proved to be of assistance in prolonging the life of the culture. In a 1–100,000 dilution, at the end of 10 days, small active amoebae were seen while bacteria were few.

With a mixture of the three dyes, gentian violet, acriflavine and phenosafranin, amoebae living in a 1–300,000 dilution were active and not reduced in size at the end of 10 days. Bacteria were few in number.

"*Acriviolet,*" a mixture prepared by Churchman and put up in tablet form, has also been used by us. It gave results similar to the acriflavine and gentian violet mixture prepared in this laboratory (table 3). Bacteria were inhibited in a dilution of 1–100,000 and 1–200,000.

A German product, "*Ubersan,*" which apparently has the properties of the flavine dyes, gave very indifferent results, no inhibition of either amoebae or bacteria occurring in a 1–200 dilution made from the original drug as bought in ampules. This preparation is sold as an antistreptococcic agent, but in the dilutions used here no such properties were noted.

TABLE 6

THE AMOEBICIDAL ACTION OF A MIXTURE OF DYES ON CULTURES OF ENDAMOEBA GINGIVALIS MIXED WITH BACTERIA

	Count of amoebae													
Dyes	Acriflavine Acridine orange		Acriflavine Sodium iodate		Gentian violet Acriflavine		Phenosafranin Acriflavine			Gentian violet Phenosafranin Acriflavine		Acriflavine Ethyl violet	
Time, hours ...	24	48	24	48	24	48	24	48	72	24	48	24	48
Dilutions:													
1-10,000													
1-20,000													
1-30,000												0*b*	0*b*
1-40,000												0*b*	0*b*
1-50,000												0*b*	0*b*
1-60,000	0*b*						0*b*	0*b*				0*b*	0*b*
1-70,000	1*b*						0*b*	20*b*				48*a*	132*b*
1-80,000	0*b*				0*b*	0*b*	2*b*	25*b*				50*a*	100*a*
1-90,000	4*b*				2*b*	0*b*	2*b*	5*b*		0*a*	0*a*	28*a*	50*a*
1-100,000	16*a*	10*a*	40*a*	128*a*	30*a*	1*a*	7*a*	23*b*	2*a*	0*a*	0*a*	8*a*	25*a*
1-200,000	0*a*	22*a*	72*a*	40*a*	32*a*	1*a*	2*a*	2*a*	1*a*	0*a*	0*a*	25*a*	60*a*
1-300,000	30*a*	38*a*	10*a*	0*a*	30*a*	1*a*	7*a*	36*a*	29*a*	2*a*	0*a*	24*a*	50*a*
1-400,000	32*a*	80*a*	20*a*	15*a*	56*a*	320*a*	10*a*	30*a*	36*a*	56*b*	192*b*	38*a*	110*a*
1-500,000	8*a*	52*a*	8*a*	16*a*			8*a*	0*a*	8*a*	184*b*	432*b*		
1-600,000										280*b*	522*b*		
1-700,000										136*b*	361*b*		
1-800,000										128*b*	160*b*		
1-900,000													
1-1,000,000													
Controls:													
a	24	368	112	660	40	224	4	371	64	8	124	80	210
b	16				5	30	7	200		128	208	20	64

Letters refer to control tubes at bottom of page.

A mixture of acid fuchsin 1–100 and sodium iodate 1–100,000 was also tried. This was found to be of about the same potency as the dye alone. There was a fair growth of amoebae in 24 hours.

It may therefore be said that the dye mixtures used were only slightly more amoebicidal than any of the constituent dyes alone, but the bacteriostatic power was so increased that practically no multiplication occurred over a 12-day period. Results are shown in table 6.

EFFECTS OF CERTAIN DILUTIONS OF DYES UPON INJURED GUM TISSUES

In an effort to determine whether or not the dyes used in the experiments just described could be tolerated by injured gum tissue in dilutions sufficient to kill amoebae, the following experiments were undertaken.

Dilutions of each dye, lethal to *E. gingivalis*, as shown in table 2, were prepared. Such solutions were made in sterile distilled water and were used at once. Healthy young rabbits were used. Each animal was anesthetized and a part of the gum tissue scarred with a sharp sterile instrument. The dye to be tested was then applied to the injured tissue, about 1 cc. being used for each animal. Animals were observed over a 3-day period and results recorded as shown in table 7.

TABLE 7

EFFECTS OF CERTAIN DILUTIONS OF DYES UPON INJURED GUM TISSUE

No. of rabbit	Dye	Dilution	Results of daily examination		
			1	2	3
1	Acridine orange	1–60,000	0	0	0
2	Acridine yellow R	1–90,000	0	0	0
3	Brilliant green	1–9,000	slight +	0	0
4	Auramine	1–60,000	0	0	0
5	Acriflavine	1–200,000	0	0	0
6	Malachite green oxylate	1–20,000	0	0	0
7	Control	Distilled water	0	0	0

At the same time, dyes in dilutions lethal to *E. gingivals* were applied to scarred normal guinea pig tissue. Animals were shaved on both sides, the skin sterilized with alcohol and scarred with a sterile instrument. The dye was then rubbed into the irritated surface with a sterile Pasteur pipette. Three dyes were used on one side of an animal and a control of sterile distilled water on the other. Results were noted for three days. It seems from these observations, that dilutions that are lethal for *E. gingivalis* are not injurious to the gum tissue of rabbits or to the skin of guinea pigs.

TABLE 8

EFFECTS OF CERTAIN DYES UPON SCARIFIED TISSUE

No. of guinea pig	Dye	Dilution	Results of daily examination		
			1	2	3
124	Acridine orange	1–60,000	0	0	0
124	Acridine yellow R	1–90,000	0	0	0
115	Naphthol blue	1–60,000	0	0	0
115	Nile blue A	1–20,000	slight +	0	0
115	Brilliant green	1–9,000	0	0	0
101	Basic fuchsin	1–9,000	0	0	0
101	New solid green 3B	1–9,000	0	0	0
101	Malachite green oxylate	1–20,000	0	0	0
	Auramine	1–60,000	0	0	0
	Acriflavine	1–200,000	0	0	0
124	Control	Distilled water	0	0	0
115	Control	Distilled water	0	0	0
101	Control	Distilled water	0	0	0

DISCUSSION

The results of the experiments given here may be divided into two groups, those dealing with the amoebicidal effects of the dyes studied and those having to do with the bacteriostatic effect upon the organisms accompanying the amoebae.

1. *Effect upon amoebae.*—The effect of dyes upon amoebae is comparable to their effect upon bacteria only in a general way. While dyes are much more toxic for amoebae in the vegetative state than they are for bacteria, there is evidence to show that those dyes most toxic for bacteria, i.e., the acridine compounds, are also the most strongly amoebicidal. The phenyl-methanes, however, have been used for bacteriostasis and also as bactericidal agents *in vitro* with some success, while as amoebicidal agents they are not active in comparison with the above-named group. Such dilutions as are toxic for amoebae are at the same time too weak to harm body tissue, if used as oral antiseptics. They might therefore be of value in eliminating amoebic

infections from gingival pockets. While the rôle of *E. gingivalis* in relation to pyorrhea has not been established, any organism of the size of the amoeba which will, on occasion, ingest red blood cells, and which, in its movement, drags a host of bacteria with it into the deeper tissues which are breaking down, must be doing considerable damage in mechanical and other ways.

Dyes have little "static" effect upon amoebae *in vitro,* a dose usually being either lethal or of no value, but with those substances which will permit the growth of the organisms over a long period of time without a change of media, there is evidence of a slight retardation of multiplication.

It has been possible to grow *E. gingivalis* for 12 days without transplant upon a modification of Boeck and Drbohlav's (1925) medium when dyes have been added in such dilution that bacterial growth was at a minimum, and to obtain good growth from subcultures at the end of this time. The optimum growth in ordinary culture media is obtained in 24–48 hours, and this is the time allowed between transplants of stock cultures which have now been under cultivation for eighteen months. The rate of division of the amoebae is undoubtedly cut down by dyes, but whether such rapid division as is ordinarily obtained in stock cultures is due to the normal cycle of development, or whether an accumulation of metabolic products of bacteria and amoebae stimulates rapid multiplication, followed by rapid death, is unknown. It is possible that if the amoebae could be grown in pure culture, they would need to be transplanted only about once in two weeks.

There is an indication that a change in pH alone does not cause the death of the amoebae. Where sodium iodate was used in certain dilutions, and the bacteria greatly inhibited, the pH dropped below that of the control tubes (table 4), and yet the growth of amoebae was better than in those tubes having a more stable hydrogen ion concentration. This seems to indicate that it is an accumulation of metabolic products other than those causing increased acidity that plays the most important rôle in the life-cycle of the amoeba as we now have it under cultivation.

2. *Effect upon bacteria accompanying amoebae.*—Selective action, while only bacteriostatic, was obtained in many cases, so that at various times cultures were comparatively free of one organism or another accompanying the amoebae. Thus, phenosafranin prevented multiplication of rod forms, ethyl violet affected the spiral forms,

acridine yellow R permitted the growth of these same forms in dilutions inhibiting other bacteria, acriflavine and sodium iodate favored cocci to the exclusion of almost all other forms, while a mixture of acriflavine and acridine orange gave complete bacteriostasis with growth of amoebae over a five-day period. We may therefore say that with the aid of dyes *E. gingivalis* has been cultivated and allowed to multiply over short periods of time with little, if any, bacterial multiplication being present in cultures, the only source of food being the medium and the relatively few bacteria carried over in transfer. Amoebae treated in this manner were normal in size and appearance. It does not seem impossible in the light of these facts, that this amoeba may ultimately be cultivated in pure culture—that is, pure culture in the bacteriological sense. Pure strains of Trypanosomes, Leishmania, and allied flagellates, the parasites of malaria and spirochaetes, are all grown *in vitro.*

The bacteriostatic and bacteriocidal power of dyes in the medium used for cultivation of amoebae is much less than where they are added to ordinary culture media for bacteria and observed *in vitro.* This is probably due to the nature of the medium, protein material being inhibitory to the action of a large number of dyes. Fairbrother and Renshaw (1922), Robertson (1925), and others have obtained results upon Protozoa which differ from those recorded here. They find the dyes used by them to be lethal to Protozoa in greater dilution than can be tolerated by *E. gingivalis in vitro,* but it must be borne in mind that these workers used fresh-water forms, such as *Paramecium* and*Enchelys,* and made their observations by placing a drop of the water containing the Protozoa on a slide, adding dye, and observing the immediate effect. In the present work, the amoebae also have been observed in an environment approximating the normal one, but, because they have been under cultivation, it has been possible to observe them over a 72-hour period.

SUMMARY

In a modification of Boeck and Drbohlav's medium, the following results were obtained by adding dyes to *Endamoeba gingivalis* in culture. Observations cover a 72-hour period.

1. The dyes used and the lethal dilution for *E. gingivalis* for each are as follows:

Acid fuchsin	below 1–200
Acid violet	below 1–1,000
Phenosafranin	
New solid green 3B	1–9,000
Brilliant green	1–9,000
Basic fuchsin	1–9,000
Malachite green oxylate	1–20,000
Ethyl violet	1–20,000
Nile blue A	1–20,000
Naphthol blue	1–60,000
Auramine	1–60,000
Acridine orange	1–60,000
Acridine yellow R	1–90,000
Acriflavine	1–200,000

2. The following dye mixtures were used and found to be toxic in dilutions approximately the same as those having acriflavine alone:

Acriflavine and acridine orange
Acriflavine and sodium iodate
Acriflavine and gentian violet
Acriflavine and phenosafranin
Acriflavine and ethyl violet
Acriflavine, phenosafranin, and gentian violet

3. The action of dyes on *E. gingivalis in vitro* is amoebicidal rather amoebastatic.

4. Of the dye groups studied, the phenyl-methanes were the least amoebicidal, and these were followed in order by the quinone-imides and the acridine compounds. Of the phenyl-methanes, the diphenylmethanes were more active than the diamino-triphenyl methanes or the triamino-triphenyl methanes. Of the quinone-imide group, the oxazines were more powerful than the azines. Acriflavine was the most powerful dye used and the strongest of the acridines, being followed by acridine yellow R and acridine orange respectively.

5. The selective action of certain dyes and dye mixtures was such that in many cases a bacteriostatic effect was obtained without toxicity to the protein of the amoeboid cell.

6. This action resulted in the prolongation of the life of the culture, so that, without transplanting or changing the medium in any way, *E. gingivalis* has remained alive and motile for 12 days.

7. There is evidence to indicate that *E. gingivalis* can live in culture with bacteria in very small numbers. Reproduction of bacteria is not necessary for the growth of *E. gingivalis* and it is considered not impossible that bacteria are unnecessary for growth, other food material being supplied.

8. Dyes alone cannot be used as a means of securing pure cultures of *E. gingivalis,* but they would be of great help in inhibiting bacteria where other means of isolation were to be used.

9. Iodates of sodium and potassium had a decidedly bactericidal effect upon organisms accompanying the amoebae *in vitro.* Cocci were unaffected.

10. Tricresol and formaldehyde used for short periods of time in strong dilutions were too active for the survival of amoebae. As disinfectants for killing cultures and for general sterilization, tricresol 1–100 and formaldehyde 1–20 are effective.

11. The dyes studied which were amoebicidal in high dilution were not injurious to the gum tissue of normal rabbits and may probably be used as oral antiseptics.

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THE SEX RATIO IN INFANT MORTALITY AS AN INDEX OF A SELECTIVE DEATH RATE

BY

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BY

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Certain general aspects of sex mortality in the first year of life have been discussed by Miss Jean Goff and the writer (1923) in a paper of which the present contribution may be regarded as, in one sense, a continuation. It was shown that, in places and in periods where infant mortality is high, the ratio of boy deaths to girl deaths is low, and conversely where there is a low rate of infant mortality the death rate of boys is relatively high. With the reduction of infant mortality that has occurred especially in recent years, there has been a marked *relative* increase of boy deaths. The sex ratio at death, or the ratio of boy deaths to girl deaths, varies with age of infants in the first year of life. For young infants it is high, but it decreases during the year. The ratio is higher for stillbirths than it is for infants dying after birth, and it is higher for abortions than for stillbirths. The available evidence shows that it is higher for early abortions than for later ones. In addition to the evidence presented in the paper referred to, I may cite the data which have recently been compiled by the United States Census on stillbirths and abortions. These have been classed according to the month of pregnancy in which they occurred, and the data for the two years now published (1922 and 1923) are summarized as follows:

Stillbirths and Abortions in the United States According to Month of Occurrence

Month	Under 2	2	3	4	5	6	7	8	9	10	Unknown
Males	9	36	307	684	1188	1795	2186	2180	7529	271	10465
Females	1	13	77	329	757	1422	1738	1852	5446	175	7975
Unknown	32	147	218	96	50	49	41	32	45	1	415
Ratio		277	399	208	157	126	125	117	138	155	9750

Following the course of sex mortality after the first year, we find a gradual reduction of the sex ratio through the early years of childhood. In some places the female death rate exceeds that of males for a few years in later childhood; and in several countries, but not in all, it is greater during the period of childbearing. After this, the male rate of mortality exceeds that of females. In the advanced age groups there are commonly many more women than men.

Differences in the mortality of the two sexes are obviously due in part to the circumstance of sex, and in part to the different conditions under which the sexes live. In some peoples the mortality of males is greatly increased by warfare. The sex ratio in the population may be profoundly modified by immigration and emigration as is conspicuously illustrated in different parts of the United States. Where many more males occur there is naturally a greater excess of males who die. Various occupations may occasion a marked increase in the death rate of one or the other sex. In the United States even lightning, which cannot be said to be a respecter of persons, kills several times as many males as females. Doubtless this is due to the fact that men are out of doors more than women. On the other hand, women are more apt to die of cancer because of the proneness of this disease to attack the uterus and the breast. The death rate from cancer is more closely associated with sex than is the death rate from lightning; but while the greater liability of men to be struck by lightning may be determined primarily by sex, because, being males, they live more in the open, this liability has no intimate connection with sex, and would not exist under altered conditions of life in which the two sexes were subjected to an equal exposure.

On account of the various conditions which modify what might be termed the natural incidence of sex mortality, I have confined my attention in this paper mainly to infants dying in the first year of life. At this time boy and girl babies are treated very much alike; their environment is much the same, and differences in habits and mode of life are not sufficiently great to have much effect upon sex mortality. Whatever disturbing conditions may occur are certainly reduced to a minimum at this time.

Differences in the distribution of deaths in a population are due in part to the accidents of environmental influence and in part to variations in hereditary endowment. Catastrophies which exterminate all people in a given area, and diseases which lead to a uniformly fatal termination, leave no opportunity for a selective death rate. In other causes of death the major rôle is played by hereditary factors. The fatality of Huntington's chorea, which is inherited as a dominant characteristic, and the frequent deaths among those afflicted with haemophilia, which is usually inherited as a recessive sex-linked character, afford striking illustrations of the importance of hereditary endowment as a deciding factor between life and death. Most causes of death apparently occupy a position somewhere between the two classes we have mentioned. Some are undoubtedly strongly selective, some mildly selective, and others not selective at all.

To ascertain the relative influences of heredity and environment in most causes of death is, in the majority of cases, a problem to whose solution we can make only the rudest approximation. Suppose we are dealing with scarlet fever or diphtheria. Obviously purely environmental factors, such as care, strongly influence the chances of recovery. Probably also differences in native vitality and differences in susceptibility (which there is some evidence for holding to be transmissible in both these diseases) may play an important rôle. It is at present doubtful to what extent these immunity reactions, which may be passed on from mother to child, depend on factors which may truly be described as hereditary; if they are hereditary, they may be quite specific in their action and have little relation to natural vigor. The problem of the inheritance of a diathesis to tuberculosis (for there is no doubt that tuberculosis tends to run in families) is tangled up with the question of direct transmission from parent to offspring in early childhood and the effects of repeated and mass infections. Many facts indicate that such a hereditary diathesis exists, but its precise rôle still remains to be specified.

The fact that many diseases are undoubtedly hereditary, and that there may be a hereditary diathesis to various diseases both constitutional and germinal, points unequivocally to the existence of a selective death rate. The arguments for the occurrence of natural selection based on the existence of hereditary causes of death are therefore on solid ground, and they constitute the best evidence we have of the action of this evolutionary factor in man. There have been attempts to demonstrate the operation of natural selection by comparing the death rates of children in successive years of life. This method has been followed by Pearson and Snow, who find that a high death rate in the first year of life is correlated negatively with the death rate for the same group of children a few years later. The inference is that if there is a high death rate in the first year a larger proportion of weaklings are eliminated, and the remainder are on the average better able to withstand the vicissitudes of future years. Of course it is a priori probable that this is what would happen. But there is, as I have elsewhere contended (1923), one circumstance which weakens the force of the argument. This is the effect of immunization which is an especially important factor in a large part of the diseases which affect early childhood. An epidemic of diphtheria or scarlet fever, for instance, even if it caused no selective mortality, would render children immune to these diseases later on, and would therefore tend to reduce the average death rate. Both a selective mortality and a direct immunity response would tend to bring about a negative correlation between the death rate in year one and the death rate in year five, and it is not evident which plays the more important rôle. This is an unfortunate complicating factor, because it tends to vitiate a method of biometric research which is otherwise of promise in revealing the actual working of natural selection.

It is not improbable that the phenomena of sex mortality may throw some light upon the selective influence of various causes of death. The fundamental differences of sex are determined by the peculiarities of the chromosome complex characteristic of males and females. They are therefore due to hereditary factors, and the greater mortality of the male may be regarded as an illustration of the action of natural selection in discriminating against one sex on the basis of its hereditary endowment. The available evidence indicates that the male is the weaker sex. If we discount the indications afforded by adult mortality as due largely to environmental factors, this conclusion is strongly supported by the phenomena of mortality in infancy and early childhood.

It has been suggested that the high mortality of male infants may be due to their larger size, larger head, and the more frequent injuries during delivery. If so, this fact should be indicated by a study of the causes which make male babies die. But the conclusion derives no support from such a study, and moreover it is strongly opposed by the fact previously mentioned that the earlier the period of ante-natal death, the higher relatively is the mortality of males. Apparently the disparity in the vitality of the two sexes is greatest at a relatively early period of uterine life, and gradually diminishes in the later periods and through the early years of childhood.

Primarily the differences in male and female mortality are due to heredity; they are an expression, directly or indirectly, of the complex of hereditary factors with which each sex starts its existence. To a certain extent perhaps the lowered vitality of males may be due to recessive sex-linked genes. Most sex-linked characters in man are recessive and are hence much more frequently found in males. A number of these, such as hæmophilia and color blindness, are distinctly deleterious traits. There may be many other factors which do not express themselves in clearly defined characteristics, but which may act as semi-lethal factors. Then there is the Y chromosome, which never occurs in the female and which therefore is never entrusted by nature with the transmission of any traits essential for the welfare of the species. In fact, it is rare that any hereditary trait is clearly dependent on factors carried by the Y chromosome. In Drosophila, whose X-chromosome carries factors for about 200 sex-linked characteristics, the Y-chromosome, which is nearly as large as the X, behaves practically as an empty vessel. With a very few exceptions it behaves in the same way, so far as known, in other forms. It may be a reservoir of a number of recessive genes having dominant allelomorphs in the X-chromosome, but normally they never have a chance of coming to expression. Ordinarily, lethal recessive genes carried by the X-chromosome or the autosomes have a better chance of being eliminated from the germ plasm. Whenever they occur in both members of a pair of chromosomes, they lead to the death of the individual and hence tend to become gradually weeded out of the stock. But the unfavorable factors in the Y-chromosome are not subject to this method of elimination. If the deleterious effect is mild in a single dose, though much more injurious in a duplex state, as so many of such factors are known to be, their elimination would be a slow process. The Y-chromosome is not essential for normal development, and it is a fact, perhaps not without significance in this connection, that it is usually smaller than the X-chromosome and frequently entirely absent. Perhaps nature is in the process of getting rid of it entirely.

It is unfortunate that we know so little of the relative vitality of the two sexes in the animal kingdom. Other things equal, we might expect that in forms in which the male is heterozygous for the factors which determine sex, the females would possess greater vitality than the males. But other things are often far from equal owing to the fact that the two sexes are often very different and lead quite different lives. Females frequently suffer from the physical drain involved in the production of large numbers of ova, or, in higher forms, from the labor of providing for the offspring. There are also differences in size, structure, food habits, and instinctive behavior which would tend to obscure any primary differences in vitality arising from the peculiar chromosome complex of the two sexes. Nevertheless there is a certain amount of evidence that in species in which the males are heterozygous for sex the female has the greater duration of life. Geiser has assembled a valuable collection of data showing the greater longevity of the female sex in fishes, insects, crustaceans, and arachnids. It is a suggestive fact that in the fruit fly, Drosophila, the course of sex mortality is much the same as it is in man. The generalization made by Geiser is that in animals in which the male is the heterozygous sex the duration of life is shorter in males than in females. The evidence that in animals in which the female is heterozygous for sex factors the greater longevity is enjoyed by the male, is less satisfactory but, as Geiser has pointed out, it is supported by Pearl's data on the domestic fowl, by some observations of Whitman on pigeons, and by some data on the Lepidoptera, although the evidence from the latter group is incomplete and somewhat contradictory.

The suggested explanation of differential sex mortality advanced by Geiser is that "The possession of two sex-chromosomes by the females of certain species of animals insures a greater longevity of the female by 'canceling out' possible mutations in the X-chromosome especially associated with lethals, while in the male there is no such 'canceling out'." This apparently is essentially the thesis advanced by Lenz to explain differential sex mortality in man. I am far from certain, however, that this differential mortality can be explained as the statistical expression of the action of unfavorable, recessive sex-linked genes. To a certain extent this is a factor but, for several reasons, I am disposed to regard sex mortality as dependent more closely on what might be termed the normal differences between the sexes than upon the sporadic occurrence of recessive mutations. The sex mortality of the lower animals yields strong support to this view. The shorter life of the male mosquito, for instance, cannot reasonably be attributed to the greater frequency of recessive sex-linked variations. The male is a different creature with different structure, rate of development, food habits, etc., and his relatively short duration of life is a perfectly normal characteristic of his sex. Many other similar cases will readily occur to every zoologist. If we view sex mortality in man in the light of phenomena presented elsewhere in nature, our conclusion receives all the support that can well be supplied by our limited knowledge in this field.

If it be true that the differential mortality of the sexes is due chiefly to heredity, then sex mortality should afford some index of the selective character of different causes of death. If a cause of death shows a high incidence of male mortality in infancy, it would probably eliminate to a considerable extent the naturally weaker infants of both sexes. Adopting this as a working hypothesis several questions naturally present themselves in this connection. Are there conspicuous differences in the mortality of male and female infants resulting from particular causes? What is the relation of sex mortality to the readiness with which the effects of death-producing agencies may be changed under a proper environment? To what extent can the causes of the differential mortality of the sexes be brought into relation with the structural or physiological peculiarities of sex, and to what extent is the differential mortality an expression of differences in general vitality? How does the sex ratio in congenital causes of death compare with the sex ratio in acquired infections or other accidents of the environment? To what extent is the lethal sex ratio associated with characteristics which can properly be described as heredity?

In order to throw light on these questions I have compiled the data on infant mortality from the United States Mortality Statistics since the beginning of the annual reports in 1900, and the more detailed information on infant mortality issued in the statistics of births since 1915. For comparison, as well as for securing a larger mass of data, a compilation was made of the records of infant mortality in the reports of the Registrar General of England and Wales from 1901 to 1919 inclusive.[1]

There is doubtless a considerable amount of error in the diagnosis of various ailments which cause infants to die. Where a combination of causes produces death, the result is attributed to only one of the factors involved. English and American statistics sometimes differ suspiciously in the number of cases assigned to some of the least fluctuating causes of death. Some of the more extensive classes of infant mortality such as "prematurity," "congenital debility," "ill-defined diseases," and "non-specified or unknown," would doubtless be distributed into other groups if an adequate knowledge of the real cause of failure were forthcoming.

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[1] For the compilation of some of the data from the U. S. Mortality Statistics I am indebted to Miss Helen Redfield, a former student in the University of California.

Changes in the classification of diseases tend to produce confusion, as well as to cause the compiler a considerable amount of inconvenience. In the United States Mortality Statistics from 1900 to 1920, group I designated as "general diseases"[2] included the common infections and also a certain number of other maladies such as rickets, scurvy, and malignant growths. In the later classification these latter diseases have been taken out, thereby making a much more logical grouping. For this reason I have removed from the corresponding group "epidemic, endemic, and infectious diseases" in table 2 the deaths due to cancer, tumor, and various deficiency diseases. Similarly meningococcus meningitis has since 1921 been removed from the group "diseases of the nervous system" and transferred to "epidemic, endemic and infectious diseases," and I have accordingly made a similar transfer of cases from "diseases of the nervous system" in the table. In other words, the present grouping in table 2 has been applied to the data compiled before the principal changes in classification were made in 1921. Since we are dealing with sex ratios, I have not considered causes of death represented by cases not sufficiently numerous to yield statistically significant results except in a few cases where the data appear to suggest some unusual condition.

The principal results of the study are embodied in the four accompaning tables. In table 1 are given the deaths with sex ratios of all persons, white and colored, in the Registration Area from 1900 to 1923 inclusive. In table 2 the deaths and sex ratios of white and colored persons are given separately since the year 1914 when separate tabulations were made on the basis of race. One reason for citing the separate data is that the general sex ratio in colored persons that die is markedly lower than in the whites. Also, the causes of infant mortality vary in the different races. Consequently in comparing the sex ratios for different diseases it is desirable to compare the relatively more homogeneous data obtained from a single race. It may be instructive to comment briefly on some of the results embodied in our tables:

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[2] Now changed to "Epidemic, Endemic, and Infectious Diseases."

TABLE 1

Deaths and sex ratios of deaths of both whites and colored population in the U. S. Registration Area from 1900 to 1923, inclusive

Cause of death	Males	Females	Ratio of male to female deaths
ALL CAUSES	1915383	1490081	128 5
I EPIDEMIC, ENDEMIC, AND INFECTIOUS DISEASES	188274	162048	116 2
1 Typhoid fever	835	789	105 8
5 Malaria	2443	2101	116 2
6 Smallpox	693	626	110 7
7 Measles	17024	13856	122 8
8 Scarlet fever	2537	2027	125 1
9 Whooping cough	38738	41727	92 8
10 Diphtheria and croup	11422	8818	129 5
11a, b Influenza	26847	20999	127 8
16 Dysentery	8586	7060	121 6
21 Erysipelas	7701	7627	100 9
29 Tetanus	6098	4233	144 1
31 Tuberculosis of the respiratory system	10145	8277	122 5
32 Tuberculous meningitis	12809	10706	119 6
33 Abdominal tuberculosis	2644	2024	130 6
57 Diabetes	238	158	150 6
II DISEASES OF THE NERVOUS SYSTEM	100763	74849	134 6
70 Encephalitis	1915	1410	135 8
78 Epilepsy	1226	863	142 1
80 Convulsions of infants	53157	38913	136 6
III DISEASES OF THE CIRCULATORY SYSTEM	16863	12602	133 8
IV ACUTE ENDOCARDITIS	2645	2021	130 8
90 Diseases of the heart	9260	7071	130 9
92 Embolism and thrombosis	617	384	160 7
94 Diseases of the lymphatics	1657	1143	145 0
V DISEASES OF THE RESPIRATORY SYSTEM	301431	233583	129 0
98 Diseases of the larynx	2441	1759	138 7
99a, b, c, d Bronchitis, acute, chronic and unspecified	50551	40774	123 9
100a, b Bronchopneumonia (including capillary bronchitis since 1921)	138591	107478	128 9
101a, b Pneumonia, lobar and undefined	98520	75345	130 7
102 Pleurisy	1629	1315	123 8
103 Pulmonary congestion	6373	4964	128 4
VI DISEASES OF THE DIGESTIVE SYSTEM	477479	379882	125 7
113 Diarrhea and enteritis	420867	339491	123 9
118a Hernia	2758	743	371 2
118b Intestinal obstruction	11195	6875	162 8
126 Simple peritonitis	2218	1574	140 9
VIII NON-VENEREAL DISEASES OF THE GENITO-URINARY SYSTEM	10805	7141	151 3
128, 129 Nephritis, acute and chronic (including Bright's disease up to 1921)	7974	5413	147 3
IX DISEASES OF THE SKIN	6529	5348	122 1
152 Furuncle	733	668	109 7
153 Acute abscess	1867	1690	110 5
X DISEASES OF THE LOCOMOTOR SYSTEM	2569	1818	141 3
XI MALFORMATIONS	110122	83180	132 4
159a Hydrocephalus	7836	6719	116 6
159b Congenital malformations of the heart	69043	48694	143 8
160 Congenital debility	170802	127792	133 6
161a Premature birth	320360	247291	129 5
161b Injuries at birth	68096	42234	161 2
179 Burns	2273	1955	116 3
194 Excessive heat	2178	1727	126 1
197–199 Homicide	1413	1233	114 6
XV ILL-DEFINED CAUSES	77131	63135	122 2

TABLE 2

Deaths and sex ratios of deaths for white and colored infants separately in the United States Registration Area 1914–1923

Cause of death	White			Colored*		
	Males	Females	Ratio	Males	Females	Ratio
ALL CAUSES	822302	625236	131 5	106611	87047	122 4
I EPIDEMIC, ENDEMIC, AND INFECTIOUS DISEASES	85726	72480	118 3	16855	14710	114 6
1 Typhoid fever	239	192	124 5	103	79	130
5 Malaria	710	555	127 9	751	627	119 8
7 Measles	7882	6394	123 3	736	569	129 3
8 Scarlet fever	855	645	132 5	26	28	92 8
9 Whooping cough	17567	18827	93 3	3248	3448	94 2
10 Diphtheria and croup	4575	3472	131 8	633	495	128 4
11a, b Influenza	19706	15669	125 7	3679	3143	117 9
16 Dysentery	2934	2447	119 9	1311	1122	116 9
21 Erysipelas	3850	3648	105 5	132	116	113 4
22 Poliomyelitis	1088	920	118 2	86	82	106 1
24 Meningococcus meningitis	1851	1227	150 8	117	97	120 6
29 Tetanus	1107	691	160 1	867	708	122 4
31–37 Tuberculosis (total)	10452	8291	126 1	1667	1462	114 0
31 Respiratory tuberculosis	3110	2397	125 9	891	824	108 1
32 Tuberculous meningitis	4723	4258	110 9	490	373	131 3
33 Abdominal tuberculosis	897	611	146 8	70	77	90 7
37a, b Disseminated tuberculosis	349	268	130 2	146	125	116 8
38 Syphilis	8225	6373	129 0	2777	2190	127 0
40 Gonococcus infection	212	274	77 4	37	44	84 1
41 Purulent infection and septicemia	883	642	137 5	53	38	139 5
43–49 Cancer and malignant tumors	268	252	106 3			
III DISEASES OF THE NERVOUS SYSTEM	20438	14626	139 7	2947	2260	130 4
70 Encephalitis	644	444	145 0			
71a Simple meningitis	3996	3987	131 7	323	246	131 3
74a Cerebral hemorrhage	861	469	132 6	82	65	126 1
78 Epilepsy	500	317	157 7	140	118	119 1
80 Convulsions	9474	6656	142 3	1960	1488	131 7
86 Diseases of the ears	1702	1238	137 4	101	70	150 7
IV DISEASES OF THE CIRCULATORY SYSTEM	6350	4696	135 2	385	290	132 8
88 Acute endocarditis	1699	1250	135 8	70	55	127 3
90 Other diseases of the heart	2969	2324	127 8	203	165	123 0
92 Embolism, thrombosis	311	189	164 5			
94 Diseases of lymphatics	1046	725	144 3	63	47	134 0
V DISEASES OF THE RESPIRATORY SYSTEM	126111	95778	131 6	18284	14748	123 9
98 Diseases of the larynx	779	596	130 7	109	90	121 1
99a, b, c Bronchitis	15526	12061	128 7	1808	1533	117 9
100a, b Bronchopneumonia (capillary bronchitis since 1921)	73739	55603	132 6	9067	7281	124 5
101a, b Pneumonia, lobar, and undefined	32518	24396	133 3	6245	5006	124 7
102 Pleurisy	921	713	129 1	74	49	151 0
103 Pulmonary congestion	1149	875	131 3			
VI DISEASES OF THE DIGESTIVE SYSTEM	181614	139379	130 3	18855	15815	119 2
113 Diarrhea and enteritis	159623	124758	127 9	16010	13625	117 5
117 Appendicitis and typhlitis	256	120	213			
118a Hernia	1142	298	383 2	209	52	401 9
118b Intestinal obstruction	5482	3350	163 6	433	312	138 9
VII NON-VENEREAL DISEASES OF THE GENITO-URINARY SYSTEM	4037	2808	143 7	556	356	156 2
128, 129 Nephritis, acute and chronic	2756	1890	145 8	391	292	133 0
IX DISEASES OF THE SKIN	3219	2625	122 2	161	114	141 2
153 Acute abscess	870	764	113 9			
155 Diseases of the bones	742	521	142 4			
XI MALFORMATIONS	62154	46893	134 7	2662	2013	132 4
159a Hydrocephalus	4002	3720	107 6	271	207	130 9
159b Congenital malformations of the heart	39286	27520	142 8	1578	1181	133 6
159c Other congenital malformations	18866	15653	120 5	813	615	132 2
XII EARLY INFANCY	298206	219108	136 1	30113	24836	121 3
160 Congenital debility, atrophy, marasmus	52098	37587	138 6	7654	6139	124 7
161a Premature birth	177804	135621	131 1	18038	15185	118 7
161b Injuries at birth	41766	25837	161 6	2016	1293	155 9
XIV EXTERNAL CAUSES	9446	7660	123 3	1775	1524	116 5
197–199 Homicide	653	578	112 9	95	80	118 7
XV ILL-DEFINED DISEASES	18563	14339	129 4	10563	8498	124 3
204 Sudden death	876	641	136 6	332	282	117 7
205b Not specified or unknown	14387	10984	131 0	10906	9161	119 0

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* In some cases the data for the colored populations have been omitted because the small number of deaths makes the sex ratios of no significance

TABLE 3

Deaths and sex ratios of deaths for infants in subdivisions of the first year in the U. S. Registration Area for births (1916–1923)

Cause of death	Under 1 month	1 month	2 months	3–5 months	6–8 months	9–12 months
All causes	M 217776	M 38096	M 29681	M 66468	M 49283	M 39239
	F 158775	F 27513	F 22133	F 50894	F 39929	F 33647
	R 137 2	R 138 4	R 134 1	R 130 6	R 123 4	R 116 6
Whooping cough	M 643	M 1351	M 1115	M 2302	M 1839	M 1860
	F 733	F 1486	F 1117	F 2210	F 2165	F 2232
	R 87 7	R 90 9	R 99 8	R 104 1	R 84 9	R 83 3
Influenza	M 1571	M 1079	M 878	M 2728	M 3088	M 2743
	F 1214	F 679	F 647	F 2008	F 2322	F 2276
	R 144 2	R 158 8	R 135 7	R 135 8	R 133 0	R 129 3
Tuberculous meningitis	M 77	M 116	M 129	M 666	M 910	M 842
	F 81	F 72	F 116	F 504	F 755	F 784
	R 95 1	R 161 1	R 111 2	R 132 1	R 120 5	R 107 4
Respiratory tuberculosis	M 115	M 134	M 141	M 464	M 496	M 487
	F 79	F 74	F 110	F 380	F 398	F 476
	R 145 5	R 167 5	R 128 2	R 122	R 124 6	R 102 3
Other forms of tuberculosis	M 61	M 90	M 114	M 253	M 216	M 206
	F 51	F 64	F 75	F 177	F 132	F 171
	R 120	R 140 6	R 152 0	R 142 9	R 163 6	R 120 5
Syphilis	M 1720	M 751	M 554	M 759	M 271	M 173
	F 1323	F 564	F 404	F 582	F 230	F 144
	R 130 0	R 133 1	R 137 1	R 130 4	R 117 8	R 120 1
Diarrhea and enteritis	M 9383	M 9517	M 9767	M 25075	M 17092	M 12146
	F 6767	F 6631	F 7175	F 18588	F 14120	F 10130
	R 138 6	R 143 5	R 136 1	R 134 9	R 121 1	R 119 9
Diseases of the stomach	M 1496	M 1038	M 761	M 1468	M 669	M 394
	F 1044	F 624	F 591	F 1076	F 536	F 360
	R 143 3	R 166 3	R 128 7	R 136 4	R 124 8	R 109 4
Bronchitis	M 2033	M 1344	M 960	M 1917	M 1352	M 891
	F 1586	F 1049	F 737	F 1482	F 1126	F 842
	R 128 2	R 128 1	R 130 2	R 129 3	R 120 1	R 105 8
Pneumonia	M 2746	M 1835	M 1457	M 4062	M 3690	M 3247
	F 2033	F 1250	F 1048	F 2839	F 2718	F 2665
	R 135 1	R 146 8	R 139 0	R 143 1	R 135 7	R 121 8
Bronchopneumonia	M 6943	M 4939	M 3630	M 9673	M 8124	M 6551
	F 4953	F 3465	F 2677	F 6961	F 6354	F 5675
	R 140 1	R 142 5	R 135 6	R 138 9	R 127 8	R 115 4
Malformations	M 26781	M 2540	M 1381	M 2047	M 988	M 613
	F 20084	F 1858	F 1018	F 1692	F 914	F 523
	R 133 3	R 136 7	R 135 6	R 120 9	R 108 1	R 117 0
Prematurity	M 93776	M 2483	M 746	M 638	M 154	M 56
	F 70305	F 2268	F 713	F 662	F 123	F 54
	R 133 4	R 109 5	R 104 6	R 96 4	R 125 2	R 103 7
Congenital debility	M 23391	M 4182	M 2955	M 3916	M 1298	M 568
	F 16862	F 2792	F 2132	F 3097	F 1093	F 492
	R 138 7	R 146 9	R 138 6	R 126 4	R 118 8	R 115 4
Injuries at birth	M 22662	M 159	M 61			
	F 14050	F 124	F 43			
	R 161 3	R 128 2	R 142			

TABLE 4

Deaths and sex ratios of deaths of infants in subdivisions of the first year in England and Wales, 1901–1919

Cause of death	Under 1 year (1901–19)	0–3 months (1901–19)	Under 1 month (1911–19)	1–3 months (1911–19)	3–6 months (1901–19)	6–12 months (1901–19)
All causes	M 1082298	M 589610	M 157958	M 78034	M 200457	M 292231
	F 840351	F 436316	F 116619	F 56468	F 159136	F 244907
	R 128.8	R 135.1	R 135 5	R 138.2	R 125 9	R 119.3
Measles	M 22703	M 656	M 90	M 223	M 1552	M 20495
	F 18883	F 491	F 72	F 162	F 1261	F 17131
	R 120.2	R 133.6	R 125 0	R 137.6	R 123.1	R 119 6
Whooping cough	M 34254	M 6912	M 322	M 2271	M 8404	M 18948
	F 37948	F 7977	F 398	F 2649	F 8581	F 21390
	R 90 3	R 86 6	R 80.9	R 85 7	R 97 9	R 88.6
Influenza	M 5496	M 1490	M 233	M 519	M 1327	M 2679
	F 3878	F 985	F 175	F 331	F 900	F 1993
	R 141 7	R 150 2	R 132 5	R 156 8	R 147 4	R 134 4
Tuberculous meningitis	M 11825	M 1072	M 43	M 310	M 3112	M 7641
	F 9285	F 659	F 29	F 206	F 2335	F 6286
	R 127.3	R 162.6	R 148 3	R 147 6	R 133 2	R 121 6
Tuberculosis, all forms	M 40100	M 6364	M 197	M 1418	M 11981	M 21755
	F 31081	F 4125	F 94	F 927	F 9241	F 17705
	R 129 0	R 130 0	R 209 5	R 152 8	R 129.6	R 122 8
Bronchitis	M 87309	M 32467	M 3779	M 8975	M 22239	M 32623
	F 67165	F 23679	F 2637	F 6580	F 16748	F 26738
	R 129 9	R 137.1	R 143 3	R 136 4	R 132 8	R 121 2
Bronchopneumonia	M 77762	M 16259	M 1488	M 5604	M 18414	M 43532
	F 57657	F 10745	F 1112	F 3738	F 13006	F 33906
	R 134.8	R 151 3	R 133.7	R 139 9	R 141 5	R 128 4
Pneumonia, lobar and undefined	M 26326	M 5534	M 626	M 1535	M 6153	M 14639
	F 19106	F 3859	F 431	F 1021	F 4308	F 10940
	R 137.7	R 143 4	R 145 2	R 150 3	R 142 8	R 133 8
Diarrhea and enteritis	M 167743	M 50480	M 4638	M 15528	M 55899	M 61364
	F 133432	F 34868	F 2702	F 10639	F 45563	F 53001
	R 125.7	R 144.8	R 171.6	R 145 9	R 122 6	R 115.8
Syphilis	M 12888	M 8823	M 2123	M 2512	M 2700	M 1363
	F 9947	F 6319	F 1375	F 1732	F 2341	F 1287
	R 129 6	R 139 7	R 154.4	R 145 0	R 115 3	R 106 0
Malformations	M 39808	M 33693	M 9826	M 3173	M 3431	M 2683
	F 31242	F 26028	F 7935	F 2271	F 2996	F 2218
	R 127.4	R 129.4	R 123.9	R 139 7	R 114 5	R 120 9
Hydrocephalus (1911–19)	M 1009	M 381	M 227	M 154	M 236	M 392
	F 772	F 357	F 241	F 116	F 183	F 232
	R 130.7	R 106 7	R 94.2	R 132 8	R 129 0	R 169 0
Malformations of the heart (1911–19).	M 6175	M 5381	M 4360	M 1021	M 430	M 364
	F 4509	F 3792	F 3086	F 706	F 398	F 319
	R 137.0	R 141.9	R 141.2	R 144.6	R 108.0	R 114.1
Premature birth	M 235707	M 222623	M 99857	M 18963	M 8811	M 4273
	F 180825	F 169977	F 75420	F 13985	F 7173	F 3655
	R 130.4	R 131.0	R 132.4	R 135.6	R 122.8	R 116.6
Injuries at birth	M 9474		M 4722			
	F 6298		F 3135			
	R 150.4		R 150.6			

INFANT DEATHS FROM ALL CAUSES—

Taking all causes of infant deaths in both the white and colored population in the Registration Area of the United States from 1900 to 1923, we find a general ratio of 128.5 boys to 100 girls. From 1914 to 1923 the death ratio for white infants is 131.5, and for colored, 122.4. Since the infant mortality for the colored population is much higher than it is for the whites, the lower sex ratio among the colored affords a striking illustration of the principle already alluded to, namely, that a low sex ratio goes along with a high infant mortality. For the same reason the much higher sex ratio since 1914 is a result of the marked decline in infant mortality which has taken place in recent years. In England and Wales the sex ratio for all causes of death from 1901 to 1919 is 128.8. This is somewhat lower than the ratio for American whites since 1914, probably for the reason that the English infant mortality rate from 1901 to 1919 averages somewhat higher than our own for a somewhat later period.

EPIDEMIC, ENDEMIC, AND INFECTIOUS DISEASES—

This group comprises the common infectious diseases of childhood, such as measles, whooping cough, diphtheria, diarrhea and enteritis, bronchitis, and pneumonia. In the present revised classification the group includes most of the maladies due to various kinds of microorganisms. More infant deaths fall into this group than into any other. As a rule the death ratio from infections is lower than the general infant death ratio. This is true for the deaths of colored and white infants from 1900 to 1923, for the deaths of white and colored separately since 1914, and for the deaths in England and Wales from 1901 to 1911. One reason for the low sex ratio for this class is the inclusion of numerous deaths from whooping cough, which is the one strongly outstanding disease which carries off more females than males in the first year. Removing the deaths caused by whooping cough from the group of general diseases, we have the ratios indicated in the following table in which the ratios of deaths from all causes are given for the sake of comparison in the column to the right:

DEATH RATIOS FROM GENERAL EPIDEMIC DISEASES, WHOOPING COUGH EXCEPTED

Colored and white (1900–1923)	124.3 as compared with 128.5
U. S. whites (1914–1923)	127.0 as compared with 131.5
U. S. colored (1914–1923)	120.7 as compared with 122.4
England and Wales (1901–1911)	126.3 as compared with 127.2

This leaves the sex ratio for general diseases below the general average, although the difference has been considerably reduced.

One important factor in determining the sex ratio for any cause of death is the period at which that cause is most prone to act. Most of the causes that act with the greatest vigor in the first few weeks of an infant's existence are likely to take off a large excess of males. The period of very early infancy is one of comparative immunity to several infectious diseases. Whether or not the cause of this immunity is to be found in the retention for a time of antibodies derived from the mother does not at present concern us. However caused, there is a considerable difference in the distribution of deaths from infections in the first year. Syphilis, which is usually contracted *in utero,* takes its heaviest toll, as might be expected, in the first month. Measles, on the other hand, does not often attack very young infants, but it is very severe during the closing months. Deaths from developmental defects, general weakness, marasmus, and prematurity, are especially frequent soon after birth, and gradually diminish in the succeeding months. In order to follow the action of several causes of death during the first year I have compiled data from the United States statistics since 1916 and those of England and Wales from 1901 to 1919. The year 1916 is the first for which the Census Bureau of the United States began to publish data on infant deaths in different periods of the first year, so that data for only seven years are available for this country. Deaths are recorded for each of the first three months separately and for three-month periods of the remainder of the year. In 1922 deaths were separately recorded for each month of the year but this useful compilation was not continued in 1923. In the British statistics there is a different grouping of age periods. An improvement in the older subdivision of the year into trimesters was made when a separate listing was given for deaths under one month.

If we follow the sex ratio through the first year we meet with a very curious fact. While in general there is a reduction of the sex ratio as we pass from birth to the end of the first year, there is an actual increase in male deaths for a short time after the first month. This relation, which soon became manifest when sex ratios were calculated for subdivisions of the year, I find has been pointed out by Dr. T. H. C. Stevenson, whose work on sex ratios has led him to conclusions similar to my own. In the proof of a forthcoming report which he was good enough to send me in the course of some correspondence on the subject, Dr. Stevenson, in commenting on one of his tables, remarks: "From these average figures [covering deaths for 1905–22] it will be seen that the excess mortality of males is almost fully manifest from the moment of birth, increasing very slightly to a maximum about the end of the first or beginning of the second month of life, after which it rapidly, and on the whole steadily, declines to comparatively small dimensions at the end of the first year."

The British and American statistics are not very well adapted for comparison on account of the different ways in which the first year is subdivided. But the data for the United States and for England and Wales agree in showing this temporary early increase in the proportion of boy deaths. In the American data the sex ratio in the second month is higher than that of the first, not only for all causes of death taken together but also for whooping cough, influenza, diarrhea and enteritis, all forms of tuberculosis except tubercular meninigitis, syphilis, pneumonia, bronchopneumonia, diseases of the stomach, malformations, and general debility. In the British statistics which we have summarized, the death ratios in months 1–3 exceed those of the first month for "all causes," measles, whooping cough, influenza, bronchopneumonia, malformations, hydrocephalus, malformations of the heart, and prematurity. Were deaths in the third month not included, probably a larger number of diseases would show this early temporary rise in the sex ratio. After the third month the reduction of the sex ratio is very decided in both British and American statistics.

What interpretation can be placed upon this early rise in the sex ratio after the first month? One fact which should be borne in mind in this connection is that considerably over half as many infants die in the first month as in all the rest of the first year. Into the first month are crowded an unduly large number of deaths from accidental causes. At this time there are, relatively to later periods, many deaths from suffocation, homicide, and other so-called external causes. The sex ratio of deaths from these sources is low. But while these influences tend to reduce the sex ratio of the first as compared with the second and later months, they afford no explanation of the rise of the sex ratio in deaths from syphilis, influenza, malformations, and other specific causes. We might say that, since a high death rate means a low sex ratio, and since the first month is preponderatingly filled with fatalities, we should expect a low sex ratio at this time. But this argument will not apply to later months of the year. There are many more deaths in the third month than in the sixth and many more in the sixth month than in the twelfth, but the sex ratio is decidedly and consistently lower in the sixth month than in the third and lower in the twelfth month than in the sixth.

Perhaps the clue to the apparently anomalous situation is to be found by regarding the phenomenon as the resultant of two opposed tendencies; first, a primary superiority of female vitality which gradually diminishes *relatively to that of males* as the age of infants increases; second, the tendency of an increase of mortality to lower the sex ratio. Throughout most of the first year the first tendency may be regarded as the predominant one, as it is before birth, but owing to the fact that so large a proportion of infant deaths are crowded into the first month the primary tendency is more nearly counteracted by the second than it is in later months.

Whether this is the true explanation or whether there is an actual *developmental* change in the relative vitality of the sexes that expresses itself for a brief period, must at present remain undecided. To the extent that the high death rate in very early life is due to non-selective or mildly selective agencies, there would tend to be a reduction of the sex ratio at this time. Considering the frailty of young infants of both sexes, it is surprising that the sex ratio of deaths in the first month is as high as it is.

The early rise in the sex ratio is all the more significant because the high sex ratio of the first month would leave a reduced proportion of boys. The sex ratio of live births is about 106 boys to 100 girls and if 6 boys and 4 girls were to perish in the first month (which is not far from the expected result) there would remain 100 boys and 96 girls subject to death in succeeding months. A high sex ratio in any month tends automatically to reduce the sex ratio of the following months, but this effect of the changing ratio of deaths is relatively slight and becomes rapidly reduced in the later three-fourths of the year. The actual changes in the sex ratio are several times greater than would be expected from this cause.

MEASLES—

The sex ratio for whites in deaths from measles is unusually low, 123.3 in the United States (1914–23), and 118.1 for England and Wales (1901–11). In deaths from measles (1900–23) the ratio for white and colored is 122.8, and for the colored (1914–23) it is relatively high, 129.3. I am inclined to think that the high ratio for Negroes is largely fortuitous since it is based on a relatively small number of cases and is somewhat less than its probable error. One reason for the low sex ratio from measles is the fact that most deaths occur in the latter half of the year when the sex ratio is reduced.

WHOOPING COUGH—

This disease presents the one conspicuous exception to the rule that more boys than girls die in the first year of life. In a previous paper (1922) I have traced the sex ratio of deaths from whooping cough in the United States through all periods of life and find that the proportion of female deaths to male deaths tends to increase with age.

Ratios of female to male deaths from whooping cough in the United States, 1900–1921, according to age

All ages	0–1	1+	2+	3+	4+	5–9	10–14	15–19	20–24	25–	Ages unknown
121	108 5	140 8	144 4	155 7	157 9	147 2	151 9	146 4	166 6	130	180

The cause of the high female death rate for this disease is unknown. Crum (1915) has shown that girls are more apt to contract the disease than boys, and of the girls who contract the disease a higher proportion die. It has been suggested that the secondary sex differences in the structure of the larynx may account for the high death rate of girls and women. I do not know of any investigation of the sex differences in the larynx of male and female infants. Dr. R. C. Scammon (1923) states that "sexual differences in the larynx appear about the third year. After this time the larynx is longer and larger in boys than in girls, and the angle between the lateral plates of the thyroid cartilages becomes more pronounced. These differences are accentuated during childhood, and particularly during puberty when the male larynx grows much more throughout than does the female."

It would hardly seem likely that the sex differences in the larynx would be great enough to cause a marked departure from the usual sex ratio in the first month. The statistics in the United States covering the first month show a sex ratio of 87.7 and those of England and Wales (1911–19) a sex ratio of 80.9. After this there is, for a brief period, a relatively larger proportion of male deaths. This reaches its maximum in the American population in the 3–5-month group in which male deaths actually preponderate over female deaths. The

British statistics show a rise to a maximum in the same period, when the sex ratio becomes 97.9, but in several of the individual years the ratio at this time exceeds 100. In the American colored population (1914–23) the sex ratio is 94.2. The number of cases in proportion to the colored population is roughly much as in the whites.

INFLUENZA—

The death rate from this disease has shown remarkable fluctuations in the last decade, the greatest change being made by the epidemic of 1918. There was a marked drop in the influenza rate for 1921, but it rose to over double this rate in the subsequent year. Deaths from influenza tend to pile up toward the latter part of the year, but not quite to so great an extent relatively as those from measles. The sex ratios for England and Wales are conspicuously higher throughout the year than those for the United States, and they are exceptionally high for the later months. Owing to the small number of cases, perhaps too much importance should not be attached to this latter circumstance.

TUBERCULOSIS—

The sex ratio in tuberculosis is not particularly high. Like most germ diseases it tends to attack infants mostly in the latter months of the year. Nevertheless, it is far from sparing the early months of infancy. Cases in which infants are born tuberculous are rare. But while commonly slow in its onset, tuberculosis carries off a certain number of babies even in the first month. Holt (1914) states that

> It was formerly believed that the disease was rare in infancy, but recent observations have shown the opposite to be the case. Statistics taken chiefly from three New York institutions where only infants and young children are received give the following figures for 382 cases of tuberculosis, the diagnosis being confirmed by autopsy in every instance. In the first year there were 160 cases, and of these 67 were under six months, ten of which were under three months of age.

In 848 autopsies on infants and children dying of tuberculosis in Vienna, those dying under three months were found to be tuberculous in 4 per cent of the cases; of those dying in 3 to 6 months of age 18 per cent were tuberculous, and of those dying in 6 to 12 months 23 per cent were tuberculous. Autopsies show the existence of tuberculosis in a considerable percentage of infant deaths from other diseases. Martha Woolstein found in 185 autopsies on children at the Babies' Hospital in New York that tuberculous lesions occurred in 1.8 per cent of cases in all infants under three months, in 12 per cent of cases under one year, and in 36 per cent of cases between one and two years.

The greater number of infant deaths from tuberculosis are caused by respiratory tuberculosis and tuberculous meningitis. The latter, although occurring at all periods, tends to be concentrated in the latter half of the year more than do deaths from the respiratory form. Perhaps for this reason the sex ratio from respiratory tuberculosis is higher than that for tuberculous meningitis.

During the period of childhood the sex ratio of deaths from tuberculosis pursues an unusual course. While most diseases continue to take a greater toll from the male sex, tuberculosis soon comes to claim a relatively larger number of victims among the girls. In England and Wales (1901–11) girl deaths begin to preponderate over boy deaths after the fifth year, but male deaths again become more numerous in the quinquennial period beginning with the twentieth year. One very natural explanation of this circumstance is that girls are more confined within doors than boys are and hence do not enjoy to so great an extent the benefits of fresh air and sunshine which are so salutary in this disease.

Deaths from tuberculosis in different age periods, England and Wales, 1901–1911

	Under 5	5—	10—	15—	20—	25—	35—	45—
Males............ ...	59239	11379	7903	16253	25934	57135	55612	45044
Females...........	49767	12526	12125	20733	23655	48472	38844	24033
Ratio...............	119.0	90.8	65.18	78.39	109.6	117.9	143.2	187.4

BRONCHITIS AND PNEUMONIA—

The acute respiratory infections constitute one of the most potent causes of infant mortality. For infectious diseases they show a fairly high sex ratio. Among the colored population, who suffer relatively more from respiratory diseases than the whites even in the first year, the sex ratio is relatively low, 123.9. In bronchitis the sex ratio is lower than it is for the pneumonias, both in the British and American statistics. For all forms of bronchitis in the United States (1914–23) it is 128.7 for the white and 117.9 for the colored population. For white and colored combined (1900–23) it is 123.9, and for infants in England and Wales (1901–19), 129.9. In the British statistics a great many more deaths are attributed to bronchitis than in the

United States statistics. Perhaps a part of this difference may result from different habits of diagnosis prevailing in the two countries. Divergent habits of diagnosis, however, could not reasonably account for all the differences observed. Another noteworthy fact is that the distribution of bronchitis through the year is quite different in the two countries, whereas the distribution of other diseases is much the same. This difference is indicated as follows:

Infant deaths from bronchitis in the United States, 1916–1923 and in England and Wales, 1901–1919

	Deaths in first 3 months	Deaths in second 3 months	Deaths in last 6 months
England and Wales	56146	38987	69361
United States	6718	2970	3675

From these figures it may be seen that over twice as many die of bronchitis in the United States during the first quarter of the year as in the second, whereas in England and Wales deaths in the first quarter exceed those of the second by less than 50 per cent. Also in the United States, deaths from bronchitis in the last six months are a little over half as frequent as in the first three months, whereas in England and Wales they are actually more numerous. Notwithstanding the fact that deaths are numerous in the first three months in the United States, the sex ratio at this time, 128.2, is not particularly high, but the relatively small number of cases should warn us not to place too much reliance on this fact.

The pneumonias, which are particularly fatal to infants, begin their ravages very early in life. In the United States more deaths from this cause occur in the first month than in any other single month. Bronchopneumonia, which in infants occurs usually as a secondary complication following other infectious diseases, shows a relatively high sex ratio associated with an early incidence. Deaths from this cause, like those from bronchitis, show a distribution during the year in England and Wales which is quite different from that found in the United States. In England and Wales the deaths are more frequent in the last six months than in the first six, and more than twice as frequent in the last six months as in the first three, whereas in the United States the deaths in the last six months are slightly fewer than in the first three. In England and Wales the deaths in the second quarter exceed those of the first, while in the United States the deaths in the first quarter greatly exceed those of the second. The differences in the distribution of deaths from other forms of pneumonia in the two countries are roughly similar to those for the distribution of bronchopneumonia. These differences are indicated in the following table:

Deaths from the pneumonias in subdivisions of the first year in England and Wales, 1901–1919 and the United States, 1916–1923

	Deaths in first 3 months	Deaths in second 3 months	Deaths in last 6 months
	Bronchopneumonia		
England and Wales	27004	31420	77438
United States	23930	14916	23923
	Pneumonia, lobar and undefined		
England and Wales	9393	10461	25579
United States	9332	6144	11026
	Bronchopneumonia and bronchitis		
England and Wales	83150	70407	146799
United States	30648	16886	27580

From the data it seems that in England and Wales deaths from pneumonia tend to crowd into the last six months of the year much more than they do in the United States. If we suppose that many deaths from bronchitis should have been classed under bronchopneumonia, or vice versa, it would not explain the discrepancy. Adding together deaths from bronchitis and bronchopneumonia, the English data show a much greater concentration of deaths in the last six months than do those from the United States.

DISEASES OF THE DIGESTIVE SYSTEM—

The sex ratio in deaths due to diseases of the digestive system is 130.3 for whites in the United States (1914–23), and 133.5 for England and Wales (1901–11). For colored people in the United States (1914–23) it is significantly lower—119.2; and for white and colored combined over a longer period (1900–23) it is 125.7. The largest number of infant deaths under this general heading is due to diarrhea and enteritis, which carry off more infants than any other kind of infection. Deaths are distributed through the year with a nearer approach to uniformity than occurs with most infectious diseases. The American statistics show a slight rise in mortality for the first three months. The English statistics do not list deaths for each of these months separately, but, since deaths under one month were separately recorded, the mortality of the last two months of the first quarter was over twice that of the first month. In the United States deaths in the second quarter are somewhat less numerous, and in England and Wales somewhat more numerous, than in the first. During the latter six months the mortality slowly declines. Apparently the maximum mortality from diarrhea and enteritis occurs somewhere near the middle of the year, but just where cannot be stated from the data in hand. The volume on Birth, Stillbirth, and Infant Mortality Statistics for 1922, which gives the mortality for each month of the first year, shows the highest mortality rate in the third month. The sex ratio in the early months is high but there is a very decided drop during the latter part of the year.

The only other class of diseases of the digestive system in which the death rate is given in the United States statistics for subdivisions of the first year is "diseases of the stomach." These are especially prevalent in the first month after which they gradually decline. The sex ratio is high in the first month (143.3), and still higher in the second (166.3), and then diminishes through the rest of the year.

Appendicitis, although not a common disease in infancy, has a ratio which is unusually high. Out of 376 deaths among American whites from appendicitis and *typhlitis,* 256 were males and 120 females, a ratio of 213.3.

Peritonitis, which often originates in appendicitis, also shows a high sex ratio. Many other cases arise from infections starting soon after birth in the umbilicus, and a certain proportion is due to tuberculosis and various other causes.

SYPHILIS—

There is no doubt that syphilis is responsible for a much larger number of infant deaths than are indicated by our vital statistics. Infantile syphilis is usually contracted *in utero* and hence begins its ravages in most cases much earlier than any other infectious disease. The death rate is particularly high in the first month and more infants die in the first three months than in all the rest of the year. The sex ratio is not so high as might be expected under the circumstances although this may be on account of the relatively small number of recorded cases. This is probably the reason why the sex ratio for the first month is 130.0 for American whites, since the ratio found in England and Wales based on a larger number of cases is 154.4. Deaths from syphilis among the colored population of the United States are relatively more common than among the whites and they show a somewhat lower sex ratio, *i. e.,* 127.0.

In considering the sex ratio in infant deaths from syphilis it must be borne in mind that this disease has already taken off a high proportion of males before birth. According to the statistics on stillbirths and abortions which have been compiled for the United States Registration Area for Births in 1922–23 there were 477 boy deaths and 349 girl deaths attributed to syphilis. Of course these data do not represent more than a very small fraction of foetal deaths resulting from syphilis, but they are perhaps sufficient to indicate the relatively high proportion of males which are eliminated by this disease before the full period of their development.

GONOCOCCUS INFECTION—

Deaths from gonococcus infection are relatively rare and they are mentioned here only because they appear to show a peculiar sex incidence. Out of 486 deaths occurring in American white infants (1914–23), 212 were males and 274 were females, the sex ratio being 77.4. In 81 deaths of Negro infants the sex ratio was 84.1. In England and Wales there are relatively much fewer deaths from gonococcus infection or else, as is much more probable, they are not reported as such, since only 32 deaths are ascribed to this cause between 1901 and 1910. The fact that in England and Wales certified causes of death are not kept from the family doubtless deters physicians in most cases from ascribing deaths due to gonococcus infection to their true cause. Dr. L. I. Dublin has shown that the proportion of deaths attributed to gonococcus infection on certificates received by the Metropolitan Life Insurance Company was more than doubled as a result of personal inquiries made to the certifying physicians. An increase of 73 per cent was found as a result of similar inquiries concerning deaths due to syphilis.

Epidemics of gonorrhoea not infrequently occur in children's hospitals and homes. According to Holt the disease "is conveyed most frequently through the medium of napkins," but other means are afforded by towels, wash cloths, underclothing, bathtubs, or the hands of a nurse who attends a larger number of infants. Doubtless more female than male infants die from this disease simply because, for purely anatomical reasons, they are more liable to become infected.

DISEASES OF THE SKIN—

The sex ratio in diseases of the skin, several of which are infections, is relatively low. *Erysipelas* has a sex ratio approaching the ratio at birth. In the newborn it is described as being "usually fatal" (Holt, 1914). The disease commonly appears in the first few weeks often starting at the umbilicus. On account of its extreme severity it might be considered as almost a non-selective cause of infant mortality. But a study of the course of its sex incidence throughout the year throws doubt on this conclusion. In England and Wales (1901–10) there were actually more deaths among girls than among boys. They were distributed as follows:

Deaths from erysipelas in England and Wales, 1901–1910

	Under 1 year	0–3 months	3–6 months	6–12 months
Males	1187	818	199	170
Females	1265	729	288	248
Ratio	93.8	112.2	69.1	68.5

Taking the data of deaths from 1911 to 1919 which give deaths under the first month we have the following trend:

Deaths from erysipelas in England and Wales, 1911–1919

	Under 1 year	Under 1 month	1–3 months	3–6 months	6–12 months
Males	590	140	264	101	85
Females	539	100	193	124	128
Ratio	109.4	140	136.7	81.5	66.4

In the United States statistics erysipelas is one of the few cases in which data are collected for subdivisions for the first year. They show a course very similar to that found in England and Wales with the same excess of female deaths in the later months.

Deaths from erysipelas in the U. S. Registration Area for Births, 1916–1923

	Under 1 year	Under 1 month	1 month	2 months	3–5 months	6–9 months	9–11 months
Males	2435	725	625	246	252	153	81
Females	2280	515	467	219	318	234	138
Ratio	106.8	140.7	133.8	112.3	79.2	65.4	58.7

The low sex ratio shown by these three sets of data, the early rise of the sex ratio after the first months, and the marked fall of the sex ratio in the latter three-fourths of the year when it exhibits a rather striking preponderance of female deaths, indicate that erysipelas is peculiar in the way in which it attacks the two sexes. After the first year deaths from erysipelas fall off very rapidly during early childhood. For a time they show a high proportion of females but the death rate soon begins to increase and to carry off more males than females, which it continues to do up to old age.

DISEASES OF THE NERVOUS SYSTEM—

Nervous diseases of infants arise from a multitude of causes. Some of the most important (meningococcus meningitis, poliomyelitis, epidemic encephalitis) are the direct results of infections and in the revised classification of causes of deaths these have been transferred to the group of general infectious diseases. In *meningococcus meningitis* the sex ratio is high—150.8 in the United States statistics (1914–23) ; 171.5 in British statistics (1901–10). On the other hand, the sex ratio in *poliomyelitis* is low (118.2 in United States whites, 1914–23). *Epilepsy* has a high ratio (157.7 in American whites; 171.5 in the British, 1901–10). One factor in the production of so-called epilepsy in infants under one year is the injury to the head inflicted by difficult labor or instrumentation and such birth injuries are much more commonly inflicted upon boys. The hereditary factor is considered to be especially important in epilepsy but the occasion for the onset of seizures in children may be indigestion, worms, and various other causes of irritation.

Convulsions—

A not inconsiderable proportion of infant deaths is attributed to convulsions. It is scarcely proper to designate convulsions as a disease; they are simply the way in which a variety of different disorders may lead to a fatal termination. The extent to which nervous instability or disorder is a factor in their production is uncertain. The sex ratio is fairly high (142.3 for whites; 131.7 for colored in United States, 1914–23; 141.0 in England and Wales, 1914–19). Most of the fatal cases occur in the first month. In the United States data the first month shows a sex ratio of 138.1; the second, 144.2; the third, 131.5; third to fifth month, 152.4; sixth to eighth, 126.8; ninth to eleventh, 112.2.

Tetanus—

The sex ratio in deaths from tetanus is unusually high, 180 for England and Wales (1901–11), and 160.1 for American whites (1914–23). The infection commonly enters through the umbilicus soon after birth, and most of the deaths therefore occur in the first few weeks. In the American statistics deaths from tetanus are not recorded for subdivisions of the first year, but the general distribution of deaths is sufficiently indicated by data from England and Wales for 1901 to 1910, which are as follows:

Deaths from tetanus in England and Wales, 1901–1910

	Under 1 year	0–3 months	3–6 months	6–12 months
Males	398	380	6	12
Females	221	217	3	1
Ratio	180.0	175.1		

According to Holt (1914) "the proportion of fatal cases is probably between 90 and 95 per cent." The relation of tetanus to sex is quite different from that of erysipelas. Both diseases are highly fatal and it might therefore be expected that both would kill the two sexes to about the same extent, but this is far from being true. That tetanus is peculiarly fatal to boys can be understood only if boys are more liable to become infected than girls. There seems to be no plausible reason for this except the greater natural resistance of the female infant. The bacillus is perhaps rather gingerly about taking hold in the beginning. But once started it pursues its relentless course without much regard for the constitution of its victims. Are naturally weak or undernourished infants more apt to *contract* tetanus?

Tetanus of the newborn is very much more prevalent among the Negroes than among the whites, as may readily be explained by differences in their surroundings. Whether the lower sex ratio (122.4) for the colored population is due to racial difference, a larger proportion of incorrect diagnoses, the greater prevalence of the disease, or some other cause, is uncertain.

Rickets—

As this is a so-called deficiency disease caused by food devoid of the requisite vitamine content, or by lack of sunshine, environmental factors are especially potent in its cause and cure. Nevertheless rickets is apparently dependent to a considerable degree upon the constitution, if not the heredity, of the infant. It is especially prone to occur in premature babies. Directly or indirectly it is responsible for a large amount of infant and of child mortality. It renders its victims liable to die of infections, and hence tends to swell the death rate from other diseases. The deaths directly attributed to this cause come mainly in the later months of infant life, yet the sex ratio is high, being 143.2 for United States whites, but only 124.5 for Negroes, among whom it is relatively much more prevalent. The British data give a still higher ratio, 158.1 The incidence and the sex ratio in subdivisions of the first year are as follows:

Deaths from rickets in England and Wales, 1901–1910

	Under 1 year	0–3 months	3–6 months	6–12 months
Males	3258	243	649	2366
Females	2006	136	400	1470
Ratio	162.4	178.7	162.2	160.9

For a later period, listing deaths under one month, we have the following data:

Deaths from rickets in England and Wales, 1911–1919

	Under 1 year	Under 1 month	1–3 months	3–6 months	6–12 months
Males	1613	26	119	318	1150
Females	1074	14	100	208	752
Ratio	150.2	185.7	119	153.0	153.0

Combining the above data we have

	Under 1 year	0–3 months	3–6 months	6–12 months
Males	4871	388	976	3516
Females	3080	250	608	2222
Ratio	158.1	155.2	159.0	158.2

So far as the data go they show that the high sex ratio of rickets continues with unusual uniformity through the subdivisions of the first year. The ratio also continues fairly high through subsequent years though showing a decline accompanying a reduction of the death rate. Data from England and Wales show the following trend:

Mortality from rickets in childhood in England and Wales, 1901–1910

	Year 1—	2—	3—	4—	Under 5	5—	10—	15—	20—
Males	3228	931	257	100	7774	102	13	13	5
Females	2458	778	223	101	5566	88	12	16	3
Ratio	131.3	119.6	115.2	99.0	139.6	116.0			

For the sake of comparison I have compiled the data on deaths from rickets for the white population of the United States since 1914.

Deaths from rickets in United States whites, 1914–1923

	Year 1—	2—	3—	4—	Under 5
Males	452	113	45	16	2008
Females	393	103	38	16	1525
Ratio	115.0	109.7	118.4	100	131.2

Altogether, the data from rickets present a rather striking evidence of the congenital inferiority of the male sex.

MALFORMATIONS—

A considerable proportion of deaths of infants have been attributed to malformations. For the most part malformations represent developmental defects resulting from specific hereditary traits or general weakness, while others are caused by the response of the developing organism to various kinds of untoward influences. Certainly malformations are produced by the most diverse causes. Syphilis, tuberculosis, and alcoholism in the mother have been considered to be, and probably are, responsible for many defects of development. One factor of importance, as shown by the studies of Mall (1921) is uterine disease, which leads to a faulty implantation of the ovum. Malformations are a not infrequent cause of abortion and the most aberrant of them are probably eliminated early in development. Mall concludes that localized anomalies occur about twelve times as frequently in early abortions as in full-term pregnancies.

According to Dr. J. A. Foote, the death rate attributed to malformations by the United States Census is much too high. The malformations which occur are, according to Foote, not the real cause of death in a considerable proportion of cases for which they are the assigned cause. Whether it is because of differences in diagnosis or for other reasons, the sex ratio of deaths from malformations is higher in the United States than in England and Wales. For the latter countries the sex ratio of deaths from congenital malformations in general is 127.4 (1901–19), for American whites (1914–23) it is 134.7, and for white and colored (1900–23), 132.4. Congenital malformations of the heart constitute over half the total cases of malformations in the American statistics and considerably less than half in the British. The sex ratio is fairly high for such deaths in England and Wales and over two-thirds of the deaths occur in the first month.

Congenital hydrocephalus, which is included under the general heading of malformations, is a defect of uncertain causation. Some cases have been attributed to syphilis, and others to tuberculosis and alcoholism, but for the most part it seems to be a disorder of development not directly resulting from any infection or toxemia. The enlarged head is present in many cases at birth and may occur some months before birth. But although boy babies have a somewhat larger head than girl babies they are not subject to a much higher death rate from hydrocephalus, at least not in the early months. Being struck by the relatively low sex ratio shown by deaths from hydrocephalus in the United States, I turned to the data from England and Wales to see how far they agreed with data from this country. The fairly high ratio there (130) is due mainly to the relatively high proportion of boy deaths in the last six months of the year, the ratio being considerably higher (169.0) for this period than that of any other cause of death. Although the number of cases is not large, the ratio for the last six months is over five times its probable error, and a similar ratio is shown for each of the several years. The sex ratio in the first half of the year is relatively low and even shows an excess of female deaths in the first months. There are no American statistics for death from hydrocephalus in different parts of the first year, but the data now available on stillbirths show a low sex ratio for this disorder—108.7, based upon 288 males and 265 females. Whether the low early sex ratio will be found in a larger series of data cannot be stated. So far as they go, the data indicate that hydrocephalus is peculiar in beginning with a low sex ratio and then exhibiting a fairly high one. The course of hydrocephalus from infancy to adolescence in the United States is as follows:

Deaths from hydrocephalus in the United States after the first year

	Year 1—	2—	3—	4—	Under 5	5–9	10–14	15–19
Males	578	232	144	85	5041	221	85	53
Females	468	181	108	68	4545	160	58	37
Ratio	123.5	128.2	133.3	125.0	110.9	138.1	146.6	143.2

HERNIA AND INTESTINAL OBSTRUCTION—

The very high sex ratio in deaths from hernia is evidently due in large part to structural peculiarities directly associated with sex. Data from England and Wales (1901–10) give 602 male and 59 female deaths under one year, a sex ratio of over 1000. But the sex ratio for a much larger number of cases from the United States is 371.2. Later in life the sex ratio in deaths from hernia becomes reversed. This change is associated with the reduced proportion of hernias of the inguinal type.

Intestinal obstruction is due to a variety of causes and is manifested in a variety of ways. Sometimes it is due to some congenital malformation of the intestines. The sex ratio is high, 183.4 in England and Wales (1901–11), 163.6 in American whites (1914–23), 138.9 in American Negroes (1914–23), and 162.8 in the American white and colored population combined (1900–23). According to Lucas (1923), "Pyloric stenosis occurs more often in male than in female babies, about 80 per cent of the cases being in males."

CONGENITAL DEBILITY—

The deaths grouped under this heading constitute one of the largest categories of infant mortality. Many deaths from congenital debility are due to parental tuberculosis, to unrecognized or unrecorded syphilis, and to many other infections of the mother. In speaking of the common condition of *marasmus,* one of the largest subdivisions of this group, Holt (1914) says that "The primary cause may be a congenital weakness of constitution which may depend upon heredity It is often seen in premature children and in the illegitimate offspring of girls of sixteen or eighteen. In the vast majority of cases, however, it depends upon two factors—the food and the surroundings." It is especially common among the artificially fed infants of the poor in tenements, and is relatively rare in children brought up in the country. "When there are not only bad feeding and unhealthful surroundings, but also an inherited constitutional vice, we have all the factors requisite to produce marasmus in its most marked form. The odds are so against the infant that the feeble spark of vitality flickers for a few months only and gradually goes out."

Unquestionably under the indefinite heading of congenital debility are lumped weaknesses due to heredity and those due to the poor condition of the mother. The latter may of course be caused either by hereditary or acquired conditions or, more properly speaking, varying combinations of both.

The sex ratio is high. For all deaths in the United States Registration Area (1900–23) it is 133.6. For American whites from 1914 to 1923 it is 138.6, and for the colored population in the same period, 124.7. The statistics for England and Wales (1900–19) give a sex ratio of 132.7. Most deaths occur in the first part of the year. In fact, in England and Wales (1911–19) somewhat over half of them fall in the first month. By following the sex ratio through the year we find, as in so many other cases, an initial rise in the sex ratio after the first month followed by a gradual decline. It is perhaps significant that the sex ratio is higher for deaths attributed to this cause in England and Wales from 1911 to 1919 than it was from 1901 to 1910. This change has gone along with a gradual diminution in the number of deaths classed in this group. In order to show this transition I have given the data for the individual years since 1911. An inspection of the data will make it apparent that, in addition to the reduction in the absolute number of deaths, the later data show also a somewhat larger proportion of infant deaths occurring in the first part of the year.

Deaths from atrophy and debility in England and Wales, 1901–1909

Deaths from 1901 to 1910

	Under 1 year	0–3 months	3–6 months	6–12 months
Males	86287	64822	13603	7862
Females	65909	46844	11854	7211
Ratio	130.9	138.3	114.7	109.0

Deaths from 1911 to 1919

	Under 1 year	Under 1 month	1–3 months	3–6 months	6–12 months	
Males	7313	3497	1971	1156	689	1911
Females	5613	2627	1441	924	621	
Males	5963	3048	1545	833	537	1912
Females	4318	2166	1037	673	442	
Males	6344	3216	1571	1014	543	1913
Females	4625	2242	1103	783	497	
Males	5855	2956	1493	884	522	1914
Females	4156	2125	1013	629	389	
Males	5307	2689	1380	754	484	1915
Females	3985	2030	930	627	398	
Males	4770	2583	1219	635	333	1916
Females	3321	1830	778	453	260	
Males	4028	2094	1048	605	281	1917
Females	3052	1620	686	470	276	
Males	3630	1974	909	496	251	1918
Females	2773	1490	690	383	210	
Males	3792	2230	928	434	200	1919
Females	2685	1595	637	310	143	
Males	47002	24287	12064	6811	3840	Total
Females	34528	17725	8315	5252	3236	
Ratio	136.1	137.0	145.1	127.7	118.7	

Dr. Newsholme (1923), in speaking of changes in death rates from congenital debility, congenital defects, and premature births, remarks that "There has undoubtedly been transference between these three groups in the course of years." As the death rate from congenital debility has decreased, the rates for prematurity and congenital malformations have increased. Deaths from prematurity have a lower sex ratio than those from congenital debility, and if there has been a transfer of cases from the latter into the former this would help to explain the rise of the sex ratio in the latter group, as well as the reduction of cases in the first.

One of the subdivisions of the group, congenital debility, namely, *icterus neonatorum*, shows consistently a high male incidence. Most of the deaths occur in the first month after which they fall off rapidly.

Deaths from icterus neonatorum in England and Wales, 1911–1919

	Under 1 year	Under 1 month	1–3 months	3–6 months	6–12 months
Males	3554	3085	416	43	10
Females	2127	1800	285	36	8
Ratio	167.1	171.4	146.0	119.4	125.0

PREMATURITY—

Deaths from prematurity naturally fall predominantly in the first month, where they show a fairly high sex ratio. In the cases from the United States (1916–23) there is a slight but not significant rise in the ratio during the second month. After the third month the sex ratio tends to be low and is often less than 100. The relatively small number of cases in the later months, however, render the data on sex ratios for this period of little value.

Premature births are the result of so many agencies that it is difficult to judge of the relative importance of constitutional and environmental factors. Among the unfavorable prenatal conditions which may result in prematurely born infants may be mentioned maternal infections, especially syphilis and tuberculosis, maternal alcoholism and other toxemias, improper or insufficient food, too frequent pregnancies, physical injuries, overwork, the use of abortifacients, and many other conditions which, by injuring maternal health, create a uterine environment unfavorable for the development of the infant. Premature births are relatively frequent where women are employed in factories or situations outside the home. They are among the direct and indirect effects of poverty and bad living conditions. English records show a rather marked increase of deaths from prematurity for several decades. From 1911 to 1919 they constituted somewhat over half the total deaths occurring under one month, while in the United States (1914–23) they were considerably less than one-half. Although deaths from prematurity, like those from congenital debility, have suffered relatively little reduction through the decrease of infant mortality, there is no doubt that better prenatal care would effect considerable improvement in both groups. Were this achieved, the sex ratio might be expected to continue to rise.

INJURIES AT BIRTH—

As might be inferred, deaths from birth injuries fall mainly in the first month, although straggling cases are reported in the latter half of the year. The sex ratio is remarkably high—161.2 for the United States, white and colored (1900–23), and 150.4 for England and Wales (1901–19). Among the Negroes (1914–23) the ratio is 155.9. The high sex ratio for injuries at birth might be attributed to the somewhat larger size and larger head of male infants, but in the light of other data it is probable that the greater weakness of male infants is a factor of even greater importance.

EXTERNAL CAUSES—

In accordance with the general conclusions set forth in this paper one would naturally infer that the sex ratio in deaths from external causes would be low—and so it is. But while the rôle of some environmental agency is emphasized in this class of deaths, the factor of native vitality plays a part even here. A glance over the subdivision ''external causes'' in our mortality statistics makes it apparent that it comprises a very miscellaneous collection of causes of death. In the case of ''poisoning by food'' or ''effects of heat'' the factor of vitality is doubtless of importance in deciding the issue between life and death. In the case of ''homicide'' the deciding factor is mainly the predilection on the part of the person committing the crime for one or the other sex. As a matter of fact the sex ratio for homicide in the first year of life is low—in England and Wales (1901–11), 105.1; United States (1900–23), 114.6; United States whites (1914–23), 112.9. Similarly in deaths from suffocation, which are mainly due to ''overlying,'' male and female infants meet their fate in about the proportion in which they appear at birth. The data for England and Wales from 1901 to 1910 are as follows:

	Under 1 year	0–3 months	3–6 months	6–12 months
Males	8652	5989	2018	645
Females	8131	5571	1893	667
Ratio	106.4	107.5	106.6	96.7

In the group classed under "violent deaths" in the British statistics we have a sufficiently large number of cases to give significant results, and they show a consistently low sex ratio throughout the year. The course of mortality from violent deaths in England and Wales (1901–10) is indicated in the table:

	Under 1 year	0–3 months	3–6 months	6–12 months
Males	11537	7595	2378	1564
Females	10711	7068	2172	1471
Ratio	107.7	107.4	109.4	106.3

The low sex ratio is in striking contrast to that of deaths from most of the other causes in which internal factors have a better chance to play a part.

SUMMARY AND CONCLUSIONS

The preceding data are, I believe, amply sufficient to make it evident that the various causes of infant mortality show consistent and often striking differences in their fatal effects upon the two sexes. To only a very minor degree can these different effects be brought into any direct relation with primary or secondary sexual characteristics. Among such exceptional instances may be mentioned hernia, which has an enormously high boy ratio; certain malformations and disorders of the genito-urinary system; gonococcus infection which, for reasons entirely anatomical, is more prone to attack female infants; and perhaps whooping cough, whose exceptional incidence *may* be associated with secondary sexual differences in the larynx. To a very minor extent also the peculiarities of early sex mortality can be reasonably explained as a result of the slightly larger size and larger head of male infants. The very high boy ratio for injuries at birth might not unreasonably be accounted for by this theory. The same interpretation might with some plausibility be extended to account for the strikingly high boy ratio in epilepsy (which is sometimes traumatic in origin) and to a certain extent also to account for deaths from convulsions and certain diseases of the nervous system. Even meningitis, poliomyelitis, encephalitis, and other infections associated with the nervous system or its membranes, might take a more severe course as a consequence of the lingering effects of birth trauma.

A comprehensive survey of the phenomena of sex mortality, however, will, I am convinced, reveal the inadequacy of this doctrine. As previously pointed out, it fails to explain the high percentage of boy deaths *in utero,* and especially the fact that boy deaths are relatively more frequent as we pass back from the period of birth. The inadequacy of the theory becomes more apparent also when we study the various causes of infant deaths. There is a general proneness of male infants to die from almost all causes to a greater extent than female infants. It is not evident why birth trauma should cause an increase of male deaths from measles, diphtheria, scarlet fever, and other common infections of the later months of infant life. Among the great multitude of babies who are born in a perfectly normal manner and who give no indication of any possible injury at birth, there is this uniformly greater boy death rate from both infectious diseases and disorders of internal origin. The high boy death rate from malformations of the heart and various other organs can certainly not reasonably be attributed to birth injury resulting from the somewhat larger size of the male infant.

The greater general proclivity of males to die of developmental disorders and weaknesses is indicative of a different ante-natal history. In the case of rickets, for instance, we have a disorder in which, notwithstanding the obvious influence of an unfavorable environment, the constitutional factor plays a very important part. The disorder is a general one, coming on usually in the later months of infancy, but nevertheless showing a high boy ratio which is continued into the subsequent years of childhood. There is no evidence that birth trauma plays any considerable part in its causation. Its foundations lie deeper in the innate constitution of the individual. The same is true for icterus,[2] diseases of the circulatory system, and other constitutional disorders of early infancy. If injuries at birth were responsible for the high percentage of boy deaths the fact would be manifested in the ways in which sex mortality in various causes of death is distributed. But it is not. On the contrary, the distribution of sex ratios in most cases stands in no probable relation to such injuries.

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[2] Many cases of icterus are the result of syphilis, but the sex ratio is markedly higher than it is for the majority of deaths from syphilitic infections.

The whole course of sex mortality in infancy, in prenatal life, as well as in subsequent age periods, is consistent with the view that the male is the inherently weaker sex, more prone on account of this weakness to developmental anomalies, congenital debility, and deaths from infectious diseases of almost all kinds. Theoretically we may explain this greater weakness as the statistical result of the influence of recessive sex linked genes. This again, I believe, is a real but minor factor. As previously explained, the greater sex mortality of the human male is probably just an incidental effect of his maleness. This is certainly true of the greater male mortality of many of the lower animals. Analogy, which is not a bad guide in such matters, would seem to indicate that the same is true of man.

Aside from the few diseases whose differential action is associated with primary or secondary sexual characters, the rank and file of disorders show not only a high average male mortality when taken together, but also marked differences in male mortality from particular causes. The explanation of these particular causes suggests many interesting problems. In part these differences are due to differences in the time of greatest incidence, since in general the earlier the onset of a disease the higher is the sex ratio of its fatal cases. This is true of infections as well as maladies due to other causes. It is true of infections because it is the constitutional factors which make the *differences* in the effect of infections on the two sexes. In general, the constitutional disorders manifest themselves relatively early and have a high sex ratio. The graver ones lead to death *in utero.* Those less grave are responsible for the majority of deaths in early infancy. But when we say that influenza and pneumonia cause a high percentage of male deaths when they attack infants soon after birth, it should be remembered that death in these cases is a function of both constitution and infection, and that it is the differences in constitution, which are greatest in early infancy, which constitute the *discriminating* factor in the sex mortality from these diseases.

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THE DORSAL AORTIC PLEXUS: A FACTOR IN THE DEVELOPMENT OF BODY WALL DRAINAGE IN THE RABBIT

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THE DORSAL AORTIC PLEXUS: A FACTOR IN THE DEVELOPMENT OF BODY WALL DRAINAGE IN THE RABBIT

BY
LEON H. STRONG

(Contribution from the Zoological Laboratory, University of California, and from the Anatomical Laboratory, Indiana University School of Medicine.)

Body wall drainage in mammals involves the successive appearance during embryonic life of three longitudinal venous plexuses each of which becomes for a time a more or less definitive trunk. Portions of each of these trunks, posterior cardinal, subcardinal (F. T. Lewis, 1902), and supracardinal (Huntington and McClure, 1907, 1920), contribute to body wall veins of the adult. These comprise the list of longitudinal channels of the dorsal body wall which have hitherto been considered as the vessels of first importance.

The purpose of the present paper is to call attention to a fourth longitudinal venous plexus designated, because of its position dorsal to the aorta and its intersegmental arteries, the 'dorsal aortic' plexus. Thus far, in my own work, the dorsal aortic plexus has been demonstrated as a component of the azygos vein, the left thoracic intersegmental veins which drain into the azygos, and the first two left lumbar veins of the rabbit. It seems probable that this plexus may be present in other mammals also.

At the suggestion of Dr. Arthur Robinson the term 'dorsal aortic' has been adopted. This term is one from the intended nomenclature of a paper soon to be published by Robinson and Reagan (1926). 'Dorsal aortic' becomes a synonym for the term 'supra-segmental,' applied to this plexus in my original abstract (1925).

This account represents a portion of a study of cervical and thoracic venous drainage in the rabbit begun some four years ago under the direction of Dr. Franklin Pearce Reagan. When the study was begun Dr. Reagan advised that especial attention be paid to intersegmental drainage and to relationships with the sympathetic trunk. I wish to acknowledge here the fruitfulness of this suggestion and others during the course of the work. Studies of cervical and thoracic drainage in the rabbit, by Howard B. Hunt and Aileen R. Andrews

(1922), students of Dr. Reagan, were constantly accessible. To these workers I am indebted. The work was aided by a grant from the Board of Research of the University of California. Although the account of the greater part of the work will appear later, and although, too, a brief abstract of the results has already been printed in the program of my final public examination for the degree of doctor of philosophy, March 13, 1925, it has seemed desirable to make the observations on the dorsal aortic plexus accessible at this time.

The development of body wall drainage in mammals is a problem concerning which there has been little agreement among many observers since 1830. In regard to the azygos veins four interpretations of development have, from time to time, appeared in the literature. The earliest, and the first propounded by Rathke (1830), was that the azygos system of veins developed from the posterior cardinal veins through transposition and persistence in the adult. Later Rathke (1838, 1861) came to the conclusion that the posterior cardinal was not the only component of the azygos vein. His descriptions are not entirely explicit. His early view was essentially held by all workers until 1893. At this time Hochstetter announced the discovery of a secondary component of the azygos. He described the azygos vein as being derived above the eighth intercostal space from the posterior cardinal; and below this level from a more dorsal component which he designated "*eine Neubildung.*" Soulié and Bonne (1905) gave an extensive account of the development of the venous system of the mole. They failed to report any system of veins contributing to the formation of the axygos other than the posterior cardinal. These observations were in agreement with Rathke's first hypothesis.

A third hypothesis of azygos formation has gradually developed as a result of the efforts of Zumstein (1897), Parker and Tozier (1898), McClure (1906), and Kampmeier (1912), an hypothesis which culminated in the work of Huntington and McClure (1907, 1920) and of Reagan (1919). Although the paper by McClure and Butler (1925) is primarily concerned with the vena cava inferior in man, the formation of the azygos vein is touched upon incidentally. The paper confirms the work on the cat by Huntington and McClure (1920). McClure and his associates brought to notice a system of longitudinal venous channels, the supracardinals, mesial and dorsal to the posterior cardinals; a system which it was maintained contributed to the azygos throughout its whole extent, except for a few segments at the proximal end, which they derived from the posterior cardinals. The uniform results obtained by this group of workers, in regard to the respective rôles of the posterior cardinal, subcardinal, and supracardinal plexuses in venous development in all forms studied, seemed to indicate a generalized ontogenetic plan which promised, with modifications, to hold good for all mammals.

Then in 1915 Sabin, who studied the veins of the pig, stated that the posterior cardinals contribute nothing to the adult azygos but that, on the contrary, the azygos veins are derived entirely from a prevertebral plexus in no way a part of the posterior cardinal system.

This statement seemed to call for further investigation. Reagan (1919) reinvestigated the pig, using a more complete series of embryos than Sabin had used. He found that the proximal portion of either azygos line might receive a variable contribution of posterior cardinal endothelium and that the upper left azygos line for a short distance is purely posterior cardinal. His results were in accord with the findings of McClure (1906) and Huntington and McClure (1920).

Despite these contributions, anatomical and embryological textbooks continued to figure modifications of Hochstetter's work on the rabbit as an hypothesis for man. Most of these were gross misrepresentations, the usual inaccuracy being the representation that Hochstetter believed the azygos vein to develop entirely from the posterior cardinal. If Hochstetter's interpretations were correct, then the rabbit is an outstanding exception in mammals in that it lacks a supracardinal system of veins. Neither Hochstetter nor those who have copied him realized the significance of his '*Neubildung,*' a structure developed from a venous plexus for which the term 'dorsal aortic plexus' (figs. 1, 4) has been adopted by Reagan and Robinson and is here used by their permission.

In view of the conflicting observations of the foregoing contributors, it became imperative that a larger number of mammals be studied in order that the general ontogenetic plan for mammals, if such existed, might be definitely determined. Since the work upon the rabbit by Hochstetter had become classic in the literature, and since subsequent observers on other mammals had been only in partial agreement with his observations, it seemed important to repeat and to extend his work. Considering the methods he had at hand and the fact that he really pioneered a complicated and difficult subject, one must conclude that he accomplished much and laid the foundation by his accurate observations for more intensive study to follow. Points which Hochstetter did not sufficiently consider were: (1) the necessarily large amount of material to be used and (2) the import-

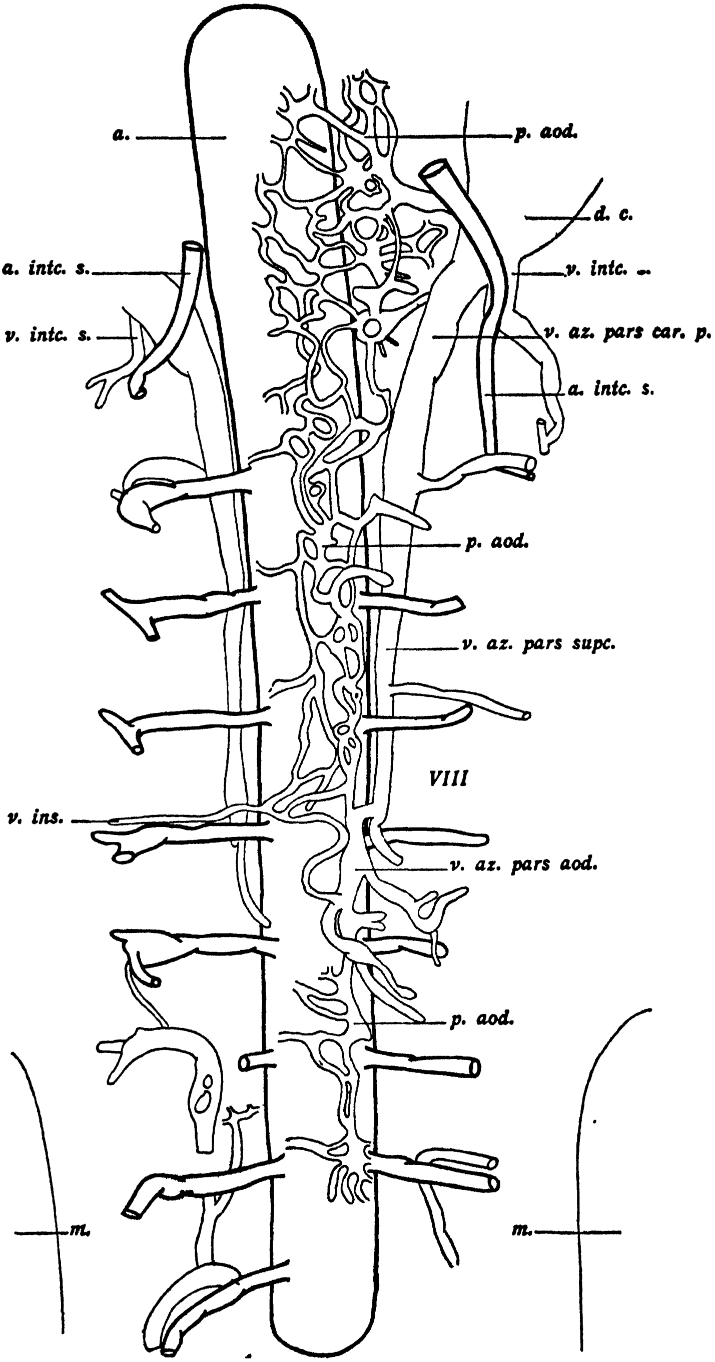

Fig. 1. Rabbit embryo, age 14 days, 18 hours. × 59. Dorsal view, from a camera lucida drawing showing the dorsal aortic plexus in apposition with the dorsal surface of the aorta. The plexus lies mesial and dorsal to the supracardinal

ance of breeding a closely timed series of embryos. Most of his stages were twenty-four hours apart. The supracardinal plexus in the rabbit, in so far as the azygos vein is concerned, appears, endures, and mostly passes in a period of eight to ten hours. None of Hochstetter's observed stages occurred at a time which would well illustrate this rapid transition if, indeed, they would reveal it at all.

In a mammal whose period of gestation is but thirty days, it proved necessary to have a closely graded series of embryos, if the critical stages of the more or less ephemeral plexuses giving rise to important longitudinal body wall veins were to be studied in their entirety. It is reasonable to presume that the failures of several observers to complete the histories of these vessels was due to the lack of such material. The individual capillaries of the supracardinal and dorsal aortic plexuses are minute. These plexuses largely degenerate in the rabbit before the middle of the fourteenth day. For adequate study it was therefore necessary to secure a series of embryos at six-hour intervals, at least during the critical stages of development. A preliminary series demonstrated the earliest critical stage to be between thirteen and fourteen days. The most important stages occur during the fourteenth and fifteenth days.

The present study was based upon a plentiful supply of rabbit embryos conceived in a series of intervals close enough to include the evanescent venous structures. It involved independent dissections numbering 190. The ages of these embryos were between the limits of twelve and twenty-three days inclusive. From this abundance of material I was able to select for description embryos which appeared to approximate, in vascular development, the norms for their respective stages. Although anomalies are most instructive, a true understanding of relationships can come only through the interpretation of such a series of norms.

All embryos used in this study were injected with India ink in the umbilical vein while their hearts were still beating. A fixative of 10 per cent formalin was found to give the best results. Clearing was done by Reagan's (1926*a*) benzyl benzoate method.

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line, which now composes the middle section of the azygos. The dorsal aortic plexus and the supracardinal component of the azygos vein are separated dorsoventrally by the intersegmental arteries. The dorsal aortic plexus has formed a strong connection with the supracardinal line, and caudally it is straightening itself out into a single vessel which forms the caudal component of the azygos vein.

a., aorta; *a. intc. s.*, arteria intercostalis suprema; *d. c.*, ductus cuvieri; *m.*, mesonephros; *p. aod.*, plexus aorticus dorsalis; *v. az. pars car. p.*, vena azygos pars cardinalis posterior; *v. az. pars aod.*, vena azygos pars aortica dorsalis; *v. az. pars supc.*, vena azygos pars supracardinalis; *v. ins.*, vena intersegmentalis; *v. intc. s.*, vena intercostalis suprema; *VIII*, eighth intercostal space.

At about fourteen days a capillary plexus (fig. 1, *p. aod.*) extends from the cervical to the lumbar region, dorsal to the aorta and its intersegmental arteries and ventral to the centra of the vertebrae. Because of its relation to the aorta this plexus will be referred to as the 'dorsal aortic plexus.' Primarily, the plexus is bilateral, but owing to the mesiality of its position it may be considered a median structure. This dorsal aortic plexus is a continuous and distinct line of venous channels. Both the supracardinal line and the dorsal aortic

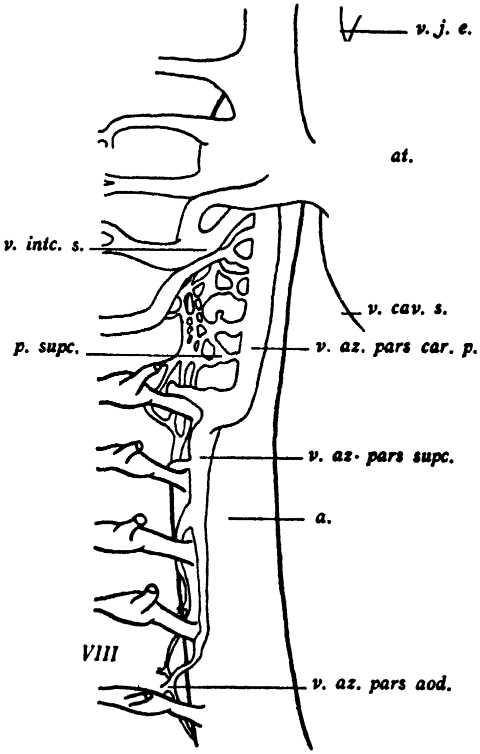

Fig. 2. Rabbit embryo, age 15 days, 1 hour. × 34. Right ventral and lateral view, from a camera lucida drawing, showing the three distinct and separate components of the azygos vein before the adult straightening out takes place. Note that the posterior cardinal segment of the azygos vein has lost most of its segmental drainage. In the adult this component receives no intersegmental veins. The supracardinal plexus is still plainly evident dorsal to the vena azygos pars cardinalis posterior and approximately in line with the vena azygos pars supracardinalis at its cephalic end. The pars supracardinalis is seen anastomosing with the pars aortica dorsalis as the latter disappears from view dorsal to the aorta and its intersegmental arteries. See fig. 3, which is a dorsal view of the same embryo.

a., aorta; *at.*, atrium; *p. supc.*, plexus supracardinalis; *v. az. pars car. p.*, vena azygos pars cardinalis posterior; *v. az. pars aod.*, vena azygos pars aortica dorsalis; *v. az. pars supc.*, vena azygos pars supracardinalis; *v. cav. s.*, vena cava superior; *v. intc. s.*, vena intercostalis suprema; *v. j. e.*, vena jugularis externa; *VIII*, eighth intercostal space.

line may be demonstrated to exist at the same time, paralleling one another in the same segments, and separated dorsoventrally by the aortic intersegmentals (figs. 1, 3). They are not therefore parts of a morphologically homogeneous system, and the derivatives of the dorsal aortic line, lying dorsal to the aortic intersegmentals, are not to be considered homologous with those derivatives of the supracardinal line which lie ventral to the aortic intersegmentals. The plexus appears most prominent in the region included between thoracic segment eight and lumbar segment one (figs. 1, 3). In this region it enlarges rapidly during the second quarter of the fourteenth day, and, at about the middle of the eighth intercostal space, it establishes

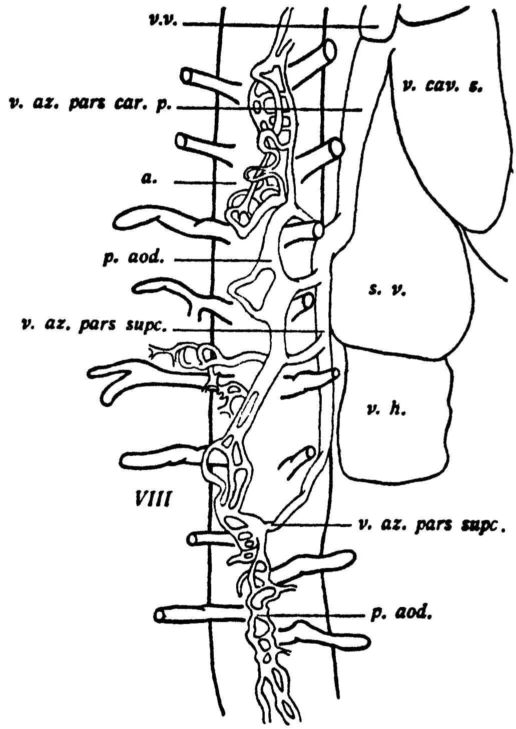

Fig. 3. The preceding embryo. × 34. Dorsal view, from a camera lucida drawing, showing the three components of the azygos vein, the dorsal aortic plexus making several anastomoses with the supracardinal portion of the azygos vein. Note the most caudal anastomosis of the vena azygos pars supracardinalis with the dorsal aortic plexus which will give origin to the vena azygos pars aortica dorsalis.

a., aorta; *p. aod.*, plexus aorticus dorsalis; *s. v.*, sinus venosus; *v. az. pars car. p.*, vena azygos pars cardinalis posterior; *v. az. pars aod.*, vena azygos pars aortica dorsalis; *v. az. pars supc.*, vena azygos pars supracardinalis; *v. cav. s.*, vena cava superior; *v. h.*, vena hepatica; *v. v.*, vena vertebralis; *VIII*, eighth intercostal space.

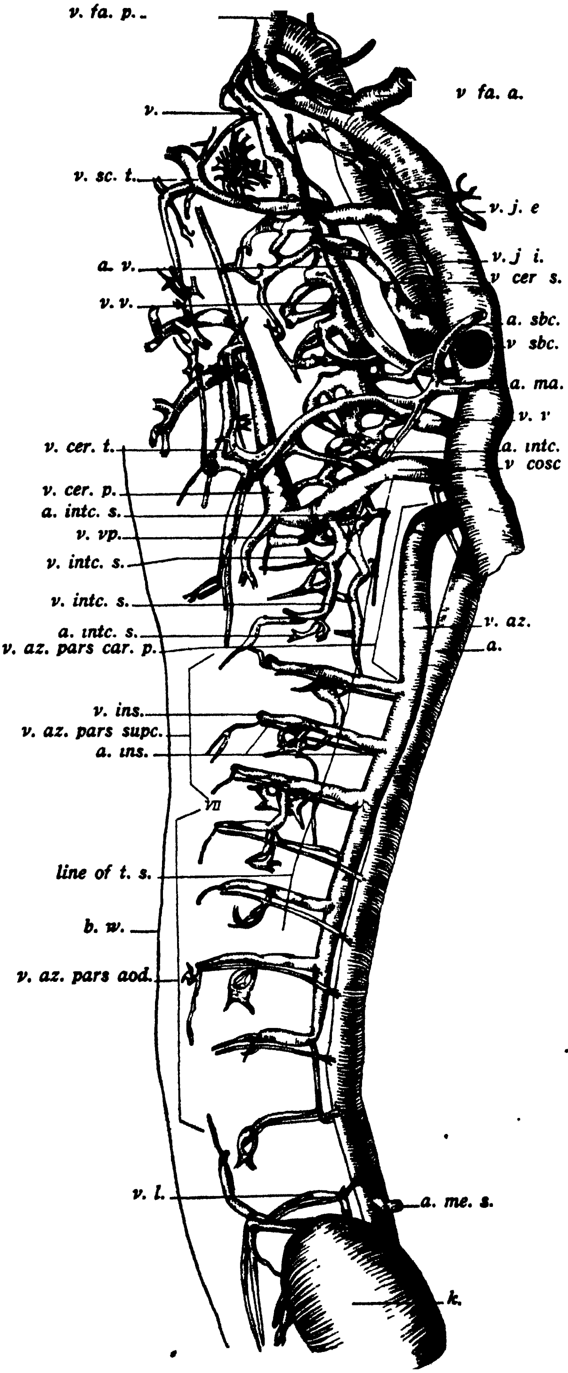

Fig. 4. Rabbit foetus, age 23 days. × 37. Ventral and right lateral view, from a camera lucida drawing, showing the cervical and thoracic drainage. The three portions of the azygos vein as it will appear in the adult have been definitely

a strong connection with the right supracardinal line (figs. 1, 2). Other strong anastomoses with the supracardinal lines are formed cephalic to the eighth intercostal space; these connections lie cephalic to and in apposition with the intersegmental arteries (fig. 3). The smaller vessels of this plexus anastomose with the small vessels of the supracardinal plexuses and with the proximal portions of the intersegmental veins of both sides of the body so that the dorsal aortic plexus acts as a series of intermediary venous channels to bridge the drainage of the right and left body walls (fig. 1).

The azygos vein in the adult rabbit consists of three parts, a pars cardinalis posterior, a pars supracardinalis, and a pars aortica dorsalis (fig. 4). The extent to which each contributes to the formation of the azygos vein shows some variation; the following description represents the average condition. The cephalic segment derived from the right posterior cardinal vein extends over intercostal spaces two to four. This portion receives no intersegmental veins since the drainage of these spaces has been taken over by the supreme intercostal veins. The middle division, derived from the right supracardinal vein, extends over intercostal spaces five to eight (fig. 4). It receives intersegmental veins from the corresponding intercostal spaces of both sides. This part of the azygos lies entirely ventral to the aortic intersegmentals. The caudal component which is derived from the dorsal aortic plexus lies entirely dorsal to the aortic intersegmentals (figs. 3, 4). It extends from the middle of the eighth intercostal space through the first lumbar space and receives the corresponding intersegmentals from both sides of the body. The union of the pars supracardinalis and the pars aortica dorsalis is effected by the persistence of a strong anastomosis between the right supracardinal and the dorsal

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established. Note that the pars cardinalis posterior drains no segments, the drainage of this region having been taken over by the vena intercostalis suprema, which lies lateral to the sympathetic trunk while the azygos vein lies mesial. The pars supracardinalis lies ventral to the aortic intersegmentals while the pars aortica dorsalis lies dorsal to the intersegmental arteries. The components of the costocervical drainage are shown. The vertebral vein is still largely plexiform.

a., aorta; *a. ins.*, arteria intersegmentalis; *a. intc. s.*, arteria intercostalis suprema; *a. ma. i.*, arteria mammaria interna; *a, me. s.*, arteria mesenterica superior; *a. sbc.*, arteria subclavia; *a. v.*, arteria vertebralis; *b. w.*, body wall; *k.*, kidney; *line of t. s.*, line of truncus sympatheticus; *v. az.*, vena azygos; *v. az. pars car. p.*, vena azygos pars cardinalis posterior; *v. az. pars aod.*, vena azygos pars aortica dorsalis; *v. az. pars supc.*, vena azygos pars supracardinalis; *v. cer. p.*, vena cervicalis profunda; *v. cer. s.*, vena cervicalis superficialis; *v. cert. t.*, vena cervicalis transversa; *v. cosc.*, vena costocervicalis; *v. fa. a.*, vena facialis anterior; *v. fa. p.*, vena facialis posterior; *v. ins.*, vena intersegmentalis; *v. intc. s.*, vena intercostalis suprema; *v. j. e.*, vena jugularis externa; *v. j. i.*, vena jugularis interna; *v. l.*, vena lumbalis; *v. sbc.*, vena subclavia; *v. sc. t.*, vena scapulae transversa; *v. v.*, vena vertebralis; *v. v. p.*, vena vertebralis posterior; *VII*, seventh intercostal space.

aortic plexuses, which is formed in the eighth intercostal space (figs. 1, 3). Cephalic to this anastomosis the dorsal aortic plexus degenerates, with the exception of those portions which contribute to the left thoracic intersegmentals. Degeneration occurs mostly during the fourteenth day. At the same time the right thoracic supracardinal line degenerates caudal to the anastomosis (fig. 2). The persisting caudal portion of the dorsal aortic plexus remains plexiform up to the seventeenth day. Since this plexus is, from its origin, dorsal to the intersegmental arteries, the adult condition remains dorsal to them (fig. 4). As indicated above, the extent to which the dorsal aortic plexus contributes to the adult azygos is variable. Hochstetter (1893, p. 587) states that the azygos vein changes from a position ventral to the intersegmental arteries to a position dorsal to these arteries at the eighth intercostal space. Of thirty adult rabbits examined during the course of this study, it was found that approximately 45 per cent confirmed Hochstetter's statement; 30 per cent showed the change to occur at the ninth space; 10 per cent at the eleventh space; 7 per cent at the tenth space. Other variations were infrequent; one of these was that in which the dorsal aortic derivative had persisted as far cephalad as the fifth intercostal space.

The dorsal aortic plexus also contributes to the thoracic and lumbar intersegmentals of the left side. Subsequent to the degeneration of the posterior cardinals, the intersegmental veins drain into the supracardinals. The dorsal aortic plexus has connections with the smaller vessels of the supracardinal plexuses and with the intersegmental veins. Thus it acts as a series of intermediary venous channels which bridge the region between the two sides of the body. This is especially significant in that the adult rabbit has no left azygos line. The intersegmental veins of the left side keep forming stronger and stronger connections with the dorsal aortic plexus so that eventually the azygos on the right side taps all of them, whereupon the left supracardinal, robbed of its tributaries, completely atrophies. Hence the drainage of the left side passes, not by the shortest path directly between the two supracardinals, but by arches dorsal to the aorta (fig. 1). Thus the proximal portions of all the left thoracic intersegmentals which drain into the azygos are derived from the dorsal aortic plexus.

The proximal portions of the first and second lumbar veins on the left side have an origin similar to that of the left intercostals which drain into the azygos. That is, the dorsal aortic plexus acts as a bridge in the region dorsal to the aorta, and out of this bridge the proximal portions of the intersegmental veins of the left side are formed. These veins cross dorsal to the aorta to terminate in the corresponding veins of the right side. Thus it is that in the adult the cephalic two lumbar veins of the left side join the corresponding two lumbar veins of the right side to form common trunks which terminate in the vena cava inferior. The remaining lumbar veins of each side, however, drain independently into the vena cava inferior. This would indicate that their origin is unlike that of the cephalic two lumbar intersegmentals.

Although other investigators have observed and figured portions of the dorsal aortic plexus it is nowhere represented as a continuous median channel, extending from the cervical to the lumbar region; nor is it anywhere considered to be a significant factor in development.

In his '*Neubildung*' Hochstetter (1893) saw only that segment of the dorsal aortic line which persists as the caudal division of the azygos vein. The limitations of his technique were responsible for his failure to see the plexus in its entirety. He could not, therefore, interpret this '*Neubildung*' as a part of a longitudinal continuous plexus which contributes not only to the azygos vein but to other structures as well.

It is significant that for the opossum McClure (1906, fig. 28, pl. 1) describes the azygos vein in two segments; one, "between its point of union with the postcava and about the middle of the tenth thoracic vertebra," which "lies dorsal to the segmental branches of the aorta"; the other, "between the tenth thoracic vertebra and its connection with the precava . . . . lies ventral to these branches." According to the present interpretation the caudal portion of the azygos vein in the opossum is a homologue of the vena azygos pars aortica dorsalis of the rabbit, and must therefore have its origin in a similar manner from a dorsal aortic plexus.

Huntington and McClure (1920) figure transverse anastomoses between the two supracardinal lines for the cat. These lie dorsal to the aorta and are designated 'intersupracardinal anastomoses.' Although these transverse anastomoses occupy a position corresponding in part to that of the dorsal aortic plexus of the rabbit, they are not joined together by longitudinal anastomoses to form a continuous median channel. However, Hochstetter (1893) observed such a channel in the 36-mm. cat embryo. He states, as a general condition for this carnivore, that from the thirteenth to the tenth thoracic vertebra the azygos vein lies dorsal to the aorta, and from the tenth thoracic vertebra to the superior vena cava it lies ventral to the aortic intersegmentals. Therefore that portion of the channel which lies dorsal to the aorta must be homologous with the vena azygos pars aortica dorsalis of the rabbit.

McClure and Butler (1925), in a study of the vena cava inferior in man, figure reconstructions of human embryos containing 'inter-supracardinal anastomoses' similar to those found in the cat. In certain regions these anastomoses are represented in a plexiform condition in which both longitudinal and transverse channels are evident. Their figures 13 and 15 show vestiges of longitudinal anastomoses which may be interpreted as the dorsal aortic plexus in man. Had injected human material been available to supplement the reconstructions, doubtless these vestiges of a longitudinal line would have been shown to be portions of a continuous though partially plexiform median channel. Of greatest significance in this connection is the atypical 45-mm. human embryo (no. 2128) of the Harvard Embryological Collection. In this embryo there is present a median longitudinal channel lying between the supracardinals, designated 'supracardinal (X).' Both the upper and lower parts of their figure 16 show that the channel is still plexiform. According to the interpretation here presented for the rabbit, 'supracardinal (X)' represents the persistent median channel of the dorsal aortic plexus. This interpretation of 'supracardinal (X)' was also arrived at independently by Reagan. In the words of McClure and Butler (1925), "As this vessel has been found to persist in the adult as a variant condition, its presence in the embryo may possibly be a regular and constant feature."

SUMMARY

The presence of the dorsal aortic plexus in the rabbit has led to an interpretation of body wall drainage different, in part, from that which has hitherto been given. There is here added the concept of a median longitudinal venous plexus, the 'dorsal aortic plexus.' This plexus lies dorsal to the aorta, and for the most part between the points of origin of the aortic intersegmentals and ventral to the vertebral centra. It extends from the cervical to the lower lumbar region and establishes frequent anastomoses with the supracardinal line and with the intersegmental veins of both sides. The dorsal aortic plexus and its derivatives are homologous neither with the posterior cardinal nor with the supracardinal systems of veins. This plexus occupies a position fundamentally different from them and may be demonstrated to exist in the same segments simultaneously with them. Hence it is not a serial homologue of either of these systems of veins.

The dorsal aortic plexus in the rabbit gives rise to the caudal component of the azygos vein, the pars aortica dorsalis. This portion of the azygos line lies dorsal to the points of origin of the aortic intersegmentals. It extends from the first lumbar space to the middle of the eighth intercostal space. Here it becomes continuous with the pars supracardinalis which lies ventral to the points of origin of the aortic intersegmentals. This middle component of the azygos vein extends as far cephalad as the fourth intercostal space where it joins the most cephalic component, the pars cardinalis posterior. The azygos vein drains into the right superior vena cava at about the level of the second intercostal space.

The dorsal aortic plexus also contributes to the proximal portions of those left thoracic intersegmental veins which drain into the azygos vein. Lumbar veins one and two on the left side are formed in a similar manner.

Since the dorsal aortic plexus exists in the rabbit, and since structures homologous with its derivatives have been described for the opossum, for the cat, and for man, it seems probable that it may prove to be a factor in body wall drainage in other mammals.

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STRUCTURAL AND FUNCTIONAL CHANGES PRODUCED IN THE GASTROPOD MOLLUSK, PHYSA OCCIDENTALIS, IN THE CASE OF PARASITISM BY THE LARVAE OF ECHINOSTOMA REVOLUTUM

BY

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INTRODUCTION

The work of Faust (1920) and of Agersborg (1924) showed that the infection of gastropod mollusks with the larval stages of trematodes is strictly harmful and is attended with more or less serious consequences for the host. The results are such that the condition can in no sense be regarded as an approach to a static relationship of no particular importance to the host. The extreme degree of infection which can be borne appears to be a measure of the great vitality of the host and its resistance in the sense of a natural or acquired immunity and not a measure of association in the nature of a biochemical truce. The warfare of host and parasite leads to the death of the former in extreme cases. Survival is perhaps largely an expression of the racial and specific immunities that may have been developed in the course of time.

Although the present paper will not deal to any extent with the immunological aspects of the problem of trematode infection in mollusks, it may be noted in passing that this is a virgin field. The investigations of Cantacuzene (1923) and many others have demonstrated the existence of immune substances elaborated on the part of a number of invertebrates in response to bacterial invasion. Erber (1923) has carried out many interesting studies on the agglutinating properties of the hemolymph of *Helix pomatia*. The general observations on the course of trematode infection suggest the existence of definite immunity factors.

The work of the first two above-mentioned authors was done entirely from a histological and cytological point of view and upon a variety of material. The work upon which the present paper is a report deals with only one species of mollusk and one species of trematode. It has been undertaken not only from the histological and cytological aspects but also from the experimental side.

Many of the findings of these two workers are confirmed in this study. In addition, many new data are reported, both microscopical and experimental, which deal exclusively with the changes produced in *Physa occidentalis* by the larvae of *Echinostoma revolutum*.

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This work was carried on under the direction of Dr. Charles A. Kofoid, whom the writer desires to thank for his many invaluable suggestions and criticisms.

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HISTORICAL REVIEW

In order to understand the pathological picture produced in any organism by a parasite, it is first of all necessary to have a clear conception of the normal condition. The gross anatomy of the gastropod mollusk is well known since a number of comprehensive monographs, as well as a great mass of minor papers, are available. The field of histology is not so well covered. The minute structure of a few forms has been described in detail. Since the histology of the gastropod mollusks, especially the pulmonates, is fundamentally the same throughout the group, differing only in minor details in specific cases, a good working basis exists for studies which would need to take this into account.

The physiology of the normal gastropod mollusk so far as known is recorded in detail in a few large works and, in restricted aspects, in many shorter papers of very diverse natures.

Least known of all are the results of parasitism in this group of animals. So far as the writer can determine only two papers have treated this field exclusively. Many authors mention in an incidental manner various possible and apparent results produced by such parasitism, but record little that would actually prove or disprove any given conclusion.

In this historical review, the writer has attempted to select the most representative literature germane to the subject. Many of the papers, especially those dealing with the physiological and pathological aspects of the subject, are pertinent only in a limited way and are passed over briefly.

ANATOMY AND HISTOLOGY

The essential features of the structure of the gastropoda were worked out by Swammerdam (1752). He described and figured the structure of the animal as a whole as well as its various organs, especially the liver. His account, as well as his figures, are substantially correct so far as they go. As would be expected from the early date of this work no histological detail is given. Subsequent workers, among them Cuvier (1817), have not materially altered this description. Cuvier (1817) in his memoir on the mollusks, included, among a large number of forms, *Lymnaea* and *Planorbis*. His text and drawings are detailed but go no farther than gross anatomy. His plate dealing with these two forms includes fifteen very clear figures. This work perhaps represents a slight advance over that of Swammerdam.

The first detailed study that gave any attention to the histological nature of a mollusk did not appear until the middle of the nineteenth century. Leydig (1850) in his monograph on *Paludina vivipara,* the river snail, showed that several types of liver cells were present. His differentiation of these was based largely on the various types of the included granules and their pigmentation. The presence of black and yellow pigments in the tissues was noted. In his figures (1850, figs. 6 and 7, pl. 12) the black pigment, as well as calcium granules, are very clearly shown in the connective tissue underlying the epithelium of the anterior part of the alimentary tube. In a later paper (1876) Leydig referred to these pigments as chromatophore granules. This later paper dealt with twelve species of gastropods, one of which was *Physa fontinalis.* Semper (1857) worked with nine species of pulmonates and his results were in essential agreement with those of Leydig. He correctly described and figured the various cells of the epithelial layer including the slime glands, as well as the general nature of the musculature and the connective tissue. The black pigment and calcium granules described by Leydig for *Paludina vivipara* are also figured by Semper for *Arion empiricorum* and *Lymnaea stagnalis.*

The nature of the cells of the so-called liver of the gastropod has been a subject for diverse opinions and heated controversy. This gland is a compound racemose structure, the tubules of which are lined with a glandular epithelium. The whole is bound together by a network of interstitial tissue in which are found branches of the hepatic artery, lacunae of the circulatory system, and the bile ducts.

The three different types of cells which compose this epithelium were designated by Barfurth (1883*a*) as ferment, liver, and lime cells. The first of these three types of cells was considered to be secretory in nature. The second type contained a large amount of yellowish granules supposedly in the nature of bile pigments. The third type of cell, the lime cell, was held by Barfurth to contain granules of tri-calcium phosphate. Biedermann and Moritz (1899) referred to the ferment cells of Barfurth as secretion cells, and to the liver cells as resorption cells. Frenzel (1883) attacked Barfurth's "lime cell" severely and attempted to show that as such it did not exist. Frenzel's work at Naples on this matter was largely based upon the microchemical behavior of the granules in the cells in question. This work was carried out on sixteen different species of gastropods—seven prosobranchs, one pulmonate, and eight opisthobranchs. His results were directly opposed to those of Barfurth. Barfurth (1883*b*), however, in answer to Frenzel's criticisms showed that the major premise of the latter was false. He himself regarded the matter of the nature of these granules as an open question.

Barfurth (1883*a*) was mistaken in stating that the lime cell is absent in water snails and present only in land snails. Faust (1920) recorded the presence of this type of cell in seven pulmonates, *Planorbis trivolvis, Planorbis guadelupensis, Physa gyrina, Physa* sp., *Lymnaea proxima, Goniobasis carinifera,* and *Physopsis africana.* The writer has identified it also beyond doubt in *Physa occidentalis.* This cell in these fresh-water pulmonates is not essentially different from that figured by Schneider (1902 and 1908) and by Meisenheimer (1912) for *Helix pomatia,* or by Prenant, Bouin, and Maillard (1904) in the hepatic epithelium of *Agriolimax agrestis,* both terrestrial pulmonates.

The structure of the emunctorium or kidney, gonad, musculature, and connective tissue is well summarized by Schneider (1902, 1908). These parts will be discussed later in special sections.

Among the works of a general morphological and taxonomic nature may be mentioned those of Simroth (1896) and Pelseneer (1897, 1906). Both these writers, in addition to much original material, have summarized a vast mass of literature on the general subject. The latest and most complete monograph on one form is Meisenheimer's (1912) treatise on *Helix pomatia,* the common garden snail. This work is not surpassed by any other in the field of anatomy and histology of the gastropod mollusks.

PHYSIOLOGY

Biedermann and Moritz (1899) referred to the hepato-pancreas of the mollusk as the "*sogenannten 'Leber'.*" Later authors, as well as some previous ones, seem to favor the term hepato-pancreas. Faust (1920) believed that the term 'liver cell' should be retained to apply to both liver and ferment cells of the previous authors. It is to be noted also that he referred to the organ as a whole as the liver.

The function of the hepatic tubules has been thoroughly studied in the mollusks. Glaser (1903) in his study on the nematocysts of nudibranchs concluded that the hepatic epithelium furnished one means of exit for harmful indigestible particles taken in with the food. In the crustacea, Weber (1880) concluded that the liver served both the function of a digestive gland and an excretory organ. Barfurth (1883*a*) as well as Frenzel (1886) ascribed this dual function to the hepatic organ of the mollusk. The "*Keulenzellen*" of Frenzel or "*cellules vacuolaires excrétrices*" of Hecht (1895) clearly indicate an excretory function. Cuénot (1899) in his paper on the excretory behavior of this organ definitely established the nature of the process. Eliot and Evans (1908) believed that, in the case of certain doridiform cladohepatic nudibranchs, the hepatic cells of excretory behavior are dropped into the lumen when distended with waste materials after the holokrinous fashion, such as that described by Garnault (1887) in the kidney of *Cyclostoma elegans.*

The digestive nature of the gland has been thoroughly established by the work of Fredericq (1878*a* and 1878*b*). He showed that this organ in *Arion rufus* and in *Octopus vulgaris* is comparable to the vertebrate pancreas, and that it does not contain bilary pigments. In this connection, Griffiths (1892, p. 108) stated that there "are no chemicophysiological reasons for saying that it also possesses a hepatic function." The work of Dor (1902), however, seems to be at variance with this conclusion. He showed the presence of urobilin in gastropods and stated that it could be transformed into a series of other pigments.

One function that this organ serves which is hepatic in nature is that of storage of food material. Biedermann and Moritz (1899), as well as earlier authors, demonstrated the storage of glycogen, fat, and calcium salts in the liver of the mollusk. Faust (1920) said that it may be possible, as Frenzel (1883) suggested, that the granules in the so-called 'lime cells' are phosphoproteins, but he is inclined to regard them as being more closely related to the basic proteins on account of their reactions to dyes. Faust, then, regarded these cells as protein-storage cells.

According to Faust (1920) there are physiologically many types of cells in the "liver" of the gastropod but cytologically only two types. One is the lime cell. Of the other he says (p. 80),

> These cells have been described as distinct types by certain authors. The ferment cells are believed to be unique in the secretion of enzymes which aid in digestion, whereas the liver cells are those in which storage of reserve food takes place. In addition, excretory functions have been assigned to each of these types. Cytologically the writer is able to recognize only one type; physiologically he is able to find many kinds of types which grade into each other almost imperceptibly. The single type of cell, which appears to contain within itself the properties of digestion, absorption and secretion, is palisade in structure, with an oval nucleus in the basal third and an elongate distal end which extends to the opening of the tubule.

The nephridium of the gastropod is well described by Schneider (1902 and 1908) and by Meisenheimer (1912). It consists of a large number of lamellae which carry the excretory cells. These cells are vesicular in nature with basal nuclei. The portion of the cell projecting into the nephridial cavity is wider than that fixed to the lamella and contains a large vacuole which may or may not contain concretions or granules. The connective tissue in the lamellae and surrounding the nephridium shows many lacunae which are in morphological connection with the circulatory system. Barfurth (1884) failed to demonstrate uric acid in the kidney of *Cyclostoma elegans*, a prosobranchiate gastropod. Strohl (1914) stated that this organ in the mussel is also free from this compound. However, the work of MacMunn (1886) clearly indicated that uric acid is present in the kidney of *Helix aspersa* and *Limax flavus*—a fact not definitely shown prior to this time. MacMunn considered this organ to exercise a renal function comparable to the vertebrate kidney. Griffiths (1892) confirmed MacMunn's work and proved the renal nature of the nephridium in *Limax maximus, Helix pomatia, Limax variegatus,* and *Arion ater.* He found urea and calcium phosphate as well as uric acid in the nephridia of these forms.

The researches of Cuénot (1892, 1894, 1900) did much to clear up the subject of excretion in the gastropoda. He (1892) summarized the topic under five heads; namely, (1) nephridial, (2) *cellules vacuolaires* of the liver, (3) *cyanophiles* of the liver, (4) the pedal gland, and (5) the Leydig cells. To the latter cells he ascribed a triple function: (1) fabrication and storage of glycogen, (2) excretion, and (3) phagocytosis. His method of injection with granular dyes (1900) proved very fruitful in elucidating the behavior of these various elements. In his note (1894) regarding the concretions in the vacuolar cells of the renal epithelium, he says (p. 450), "Ces masses de concretions ne sont rejetées qu'à des intervalles éloignés, quinze jours, un mois et plus, soivant l'activité de l'animal; durant toute la période d'hibernation (cinq mois) les Helix ne rejettent absolutement rein."

The subject of digestion in the gastropods is treated at length in Jordan's (1913) monograph on this process in the invertebrates. Since the present paper will not touch on this phase of the physiology of the snail, only brief mention will be made of it.

Bernard (1853) pointed out the existence of reducing sugars in the livers of *Limax flava* and *Helix pomatia.* He gave an interesting account of the elaboration of sugar and its relation to the secretion of the 'bile' and the process of absorption. The presence of glycogen in various parts of the body was shown by Barfurth (1885) for *Limax variegatus* and *Helix pomatia.* Blundstone (1885) recognized this substance as a consistent of the connective tissue; while Hammersten (1885) substantiated the findings of both Barfurth and Blundstone. The reports of later authors, among them Biedermann and Moritz (1899), Jordan (1913), Faust (1920), and Agersborg (1924) have amply established the presence of glycogen and fat in the tissues of the snail. That of Jordan gave detailed quantitative data for healthy animals; that of Faust in particular elucidated the behavior of these substances in parasitism. These papers are cited more in detail later.

Concerning the mechanics and physiology of respiration in the mollusks, much has been written, which need not concern us here. Quantitative data on the respiratory exchange are relatively scarce. The most important sources may be briefly mentioned. Thunberg (1905*a*) reported respiratory quotients for *Limax agrestis*; Hesse (1910) for *Helix pomatia;* Meyer (1925) for *Bulimus detritus* and *Cyclostoma elegans.* Such papers will be referred to when the respiratory quotients of *Physa occidentalis* are discussed.

PATHOLOGY

An extensive and detailed literature deals with the effects of parasitism upon man and his domesticated animals. The pathology of such infections as that of the common liver fluke and the schistosomes has been very thoroughly studied. Such studies are of interest in a comparative sense; but, for the purposes of the present paper, it is not necessary to summarize them here.

Ward (1907) pointed out that the effects of parasites on the host may be mechanical, morphological, and physiological. A single parasite may have no effect; it is the multiplication of parasites that is to be feared. The location of the parasite in the tissues is also a great factor in determining the degree of effect. Ward also pointed out that the most common structural local reaction of the host to the parasite is the formation of a cyst about the invader by the proliferation of the tissues of the host, particularly the connective tissue. Actual destruction of tissues might occur. The physiological processes are certainly very profoundly modified and the growth of the host is sometimes limited. In many cases sex development is inhibited. These generalities of Ward are applicable to parasitism in vertebrates and invertebrates alike.

Concerning the results of the parasitism of mollusks by larval trematodes, Lebour (1911, p. 423) wrote,

> The presence of sporocysts and rediae certainly injures the molluscan host. The outer appearance of the digestive gland is usually enough to show if it is inhabited by these parasites. It looks unhealthy and is a grey, sickly yellow or a peculiar unnatural orange. It is generally completely riddled with the sporocysts or rediae which feed upon its substance. If the gonad is infected it is quite destroyed.
>
> On the other hand encysted cercariae seem to do little or no harm to their hosts even when present in great numbers.

Cort (1915) recorded that the color of the digestive gland of the mollusk is often changed by the pigmentation of the sporocysts or rediae that it may contain. Likewise, Hurst (1923, p. 7) said, "The viscera of an infected snail has a sickly appearance and can often be told at a glance. In fairly heavy infection, it is often mottled and of a whitish or a dirty color." Similar citations may be multiplied, but they deal only incidentally with observations of a general nature. Sewell (1922), in addition to remarks similar to the foregoing noted that infection is fatal in many cases and that slight changes in the environment may cause the death of the snail. He found snails, the livers of which appeared abnormal, suggesting infection, but no parasites were noted. Since in these cases there was evidence of fatty degeneration and no gonads were present, he concluded that a possible condition of recovery had supervened. The physiological stimulus to the miracidia by the snail might possibly be altered under such circumstances and confer an immunity to attack, an immunity which would allow cercariae to enter and encyst but would bar the entrance of free-swimming larvae. Such immunity, he suggested, might be hereditary and a physiological generic character. Like Lebour (1911), Sewell regarded the cysts as apparently of no pathological significance.

According to Faust (1917) parasitism of the gastropod by larval flukes results in serious mechanical and chemical disturbances. There is marked distension of the organs and piercing of the tissues of the host by the armature of the worms. Fibromata result and an attempt is made to isolate the parasites by encapsulation. The parasites are found in the intercaecal spaces. No infection was so light that it produced no effect. In cases of heavy infection, the damage resulting when the cercariae bored their way out was considerable. Tissue degeneration, the accumulation of fatty bodies, vacuolization, sloughing of necrotic cells into the lumen, and cytolysis and karyolysis were characteristic effects that were observed by Faust in the hepatic organ of the snails with which he worked.

In his later paper (1920) Faust undertook a more thorough analysis of the effect of infection with larval trematodes on the gastropod host. This analysis was confined entirely to the digestive gland and records many cytological and histological changes of interest. These will be taken up in the section of this paper that deals with the histological and cytological nature of the response to parasitism on the part of the hepato-pancreas. The same will be done with the data recorded by Agersborg (1924). The latter author, however, deals at length with a granular reaction product which he believes to be associated with the cycle of parasitism. He also discusses the question of an "antidote" to the products of the parasite and interprets these granules in that light.

MATERIAL AND METHODS

The gastropod used in this study was *Physa occidentalis* Tryon. These animals were obtained in abundance at all seasons of the year from Stow Lake and the chain of lakes in Golden Gate Park, San Francisco, and from Strawberry Creek on the University campus in Berkeley. A very large percentage of those obtained from Golden Gate Park were infected with the various larval stages of the trematode *Echinostoma revolutum* Froelich. The anatomy of the adult form is well known (Looss, 1899; Lühe, 1909; Dietz, 1910) and need not be discussed. This species of trematode is cosmopolitan; it has been reported from California (Johnson, 1920), Australia (Johnston, 1913, 1916; Nicoll, 1914); and from various places in Europe (Looss, 1899; Lühe, 1909). The life-history of *Echinostoma revolutum* has been worked out by Johnson (1920). Briefly stated this cycle consists of the adult worm which infests the intestines of various ducks and geese, the free-swimming miracidium, and the daughter redia, mother redia, cercaria, and cyst stages; the last four are all found in *Physa occidentalis*. Lühe (1909) described a cercaria, *Cercaria echinata*, which he supposed to be the larva of *Echinostoma revolutum*.

Various methods were used to prepare material for histological and cytological examination. All snails were carefully removed from their shells before fixation. This removal was so carefully performed that the animals in many cases crept around in the dish as though nothing had happened to them. Fixation was in hot (60° C.) Bouin's fluid, Zenker's fluid, or Schaudinn's fluid. Of these fixing agents, Bouin's fluid seemed to give the best results for general histological and cytological purposes. Material fixed in the above reagents was stained with Heidenhain's haematoxylin and counterstained with eosin.

For cytological details other than nuclear structure, special methods were used. The Mann-Kopsch osmium tetroxide method was tried for the Golgi bodies, mitochondria, and fat bodies. This followed a preliminary fixation in osmo-sublimate (Lee, 1924). The most satisfactory fixing agent to be followed by the Mann-Kopsch osmic treatment proved to be the fluid devised by Bell (1926), containing two parts saturated aqueous solution of picric acid, one part of saturated aqueous solution of bichloride of mercury, and one part of *pure* 40 per cent formalin. Cajal's method as outlined by

Pappenheimer (1916) was also used. The most successful method for the demonstration of the Golgi bodies and mitochondria seemed to be the modification of Cajal's technique that was reported by Da Fano (1920). Differentiation of the various structures of a lipoid nature (Golgi bodies, mitochondria, fat bodies) was based on the data given by Lee (1924) and by Gatenby (1919).

Fat was stained in frozen sections by the Herxheimer Scharlach R method after formalin fixation as described by Mallory and Wright (1921). The osmicated material, however, gave the better picture of this substance.

Material intended to show the relations of glycogen was fixed in absolute alcohol and sectioned in celloidin. Best's carmine countered with alum haematoxylin followed this treatment. Better results by far were obtained by the iodine method of Gage (1906). In this method, the paraffin sections are spread on the water bath on the special iodine solution instead of on water. The paraffin was removed with xylol in the usual way and the preparations mounted in vaseline. A modification of Kofoid's (1919) iodine-eosin stain was successfully employed in the same way as that of Gage cited above. This modified solution is made by adding to two parts of 5 per cent aqueous solution of potassium iodid, saturated with iodine, one part of absolute alcohol saturated with eosin. The paraffin was removed from the sections with xylol saturated with eosin. Mounts were made in balsam but had the disadvantage of not being permanent.

Two general techniques were employed for certain purposes to study material directly either with or without fixation. These were the moist smear method and the compression method. For the latter, the ordinary compressor with a thin round coverglass in the upper movable part as used for the examination of muscle tissue for *Trichinella* was employed. Smears were stained either with or without fixation in hot Schaudinn's fluid by Wright's and Giemsa's methods for the phagocytic cells. Unfixed dry smears were used to study the pigment granules. These granules were bleached by Mayer's chlorine method (Schmorl, 1909), then followed by a variety of stains and microchemical tests.

In studying the respiratory exchanges of *Physa occidentalis* in health and parasitism, two different methods were utilized. The first of these was a modification of the phenolsulphonphthalein method of Child (1919) for measuring the carbon dioxide output of *Planaria dorotocephala.* The snails were weighed 'dry'; that is, they were rolled on an absorbent towel and allowed to remain with the foot in contact with the towel until the exterior of the shell was perfectly dry. They were then weighed on an analytical balance to the nearest tenth of a milligram and placed individually in small test tubes each containing five cubic centimeters of water of a known pH. The tubes were then corked and sealed with paraffin. Control tubes were treated similarly. At the ends of varying periods (about four hours) one-fourth cubic centimeter of the standard indicator solution was introduced into each tube and the results read to the nearest 0.1 (e.g., 7.0, 7.1, 7.2, etc.) in the range of color. The total volume of fluid and the concentration of the indicator were the same in both the tests and in the standards.

The second method for measuring the metabolic rate was by means of a Thunberg-Winterstein microrespirometer (Thunberg, 1905*b*; Winterstein, 1912). This apparatus was successfully employed by Ball (1925) to measure the oxygen consumption of *Paramecium.* Essentially, this apparatus consists of two small glass chambers connected by a glass tube with a two-millimeter bore. Each chamber has a capacity of about three cubic centimeters and is closed with a ground glass stopper carrying a small glass cup which projects downward into the upper part of the chamber. The snail to be tested, after weighing, is placed in one chamber; both chambers are then filled to a uniform level with water from the aquarium. The carbon dioxide given off is absorbed by concentrated $Ba(OH)_2$ solution placed in the two cups. The difference in the amounts of oxygen absorbed in the two chambers represents that used by the snail. This may be conveniently measured by observing the movement of an oil droplet in the connecting tube. The apparatus as a whole is suspended in a water bath.

The method of Lund (1919) for the determination of small amounts of carbon dioxide by direct titration of the $Ba(OH)_2$ solution was modified to suit the Thunberg-Winterstein apparatus. By this combination, both carbon dioxide elimination and oxygen consumption could be measured with one operation.

Quantitative determinations of the fat contents of healthy and parasitized snails were attempted by a micro-adaptation of the Babcock method; this proved too cumbersome to be practical and was abandoned in favor of ether extraction. Weighed quantities of snails were thoroughly ground in a mortar with clean quartz in repeated changes of ether. The total solution together with risings was evaporated on a weighed filter paper. This was allowed to dry two hours and again weighed for increase in weight.

Reducing sugars were estimated by the method proposed by Benedict (1911). The procedure followed was identical to that described by Benedict except that the amount of the reagent was reduced to that necessary to be completely decolorized by one milligram of sugar. Aqueous solutions carrying the sugar content of the snails were prepared by grinding in the mortar in the same way as that used for extracting the fat.

The total nitrogen excreted was measured by a modification of the Folin-Farmer (1912) micro-Kjeldahl test. Snails were tubed as for the indicator method noted above and left twenty-four hours. At the end of this period, they were removed and the contents of the tubes, together with controls, were separately digested by the sulphuric acid mixture. The distillate, after the addition of the alkali, was received directly into 200 cubic centimeters of indicator (brom thymol blue) of known pH.

Wohlgemuth's (1908) procedure was used to test the amylolytic activity of extracts of the snails. The phagocytic activity of the amoebocytes was studied by injecting living *Bacillus tuberculosis,* avian type, into the snails by means of the micro-injection apparatus described by Hurst (1925). This apparatus was also used to inject living cercariae of *Echinostoma revolutum* into *Lymnaea* sp.

GENERAL OBSERVATIONS ON BOTH HEALTHY AND PARASITIZED PHYSA OCCIDENTALIS

Before entering upon an exposition of the specific changes produced in *Physa occidentalis* by the larva of *Echinostoma revolutum,* a few observations of a general nature will be set forth. For convenience these have been grouped under four heads.

Appearance and Behavior

Healthy snails are usually much darker in color than unhealthy ones. Often they are nearly black because of the large amount of pigment in the mantle and in the dorsal epidermis of the foot and head. The creeping sole, although pigmented, is not so dark as the upper parts. In parasitized *Physa* the color is in many cases greatly modified. The amount of modification apparently is directly proportional to the degree of infection and is due to at least two causes. These are: first, the reduction in the pigment of the snail, and second, the appearance of the pigment of the parasite itself. The rediae vary to a very considerable degree in color. This variation ranges from the almost transparent condition to heavy pigmentation in which the parasites are dark brown. According to Johnson (1920) this is perhaps an expression of the age of the rediae; the younger being lighter in color, the older being darker. More commonly the color is light orange. The most efficacious source of color change, however, seems to be the reaction of the mechanism of pigment production in the host rather than the pigment of the parasite. That of the parasite produces a more or less local effect in the hepato-pancreas, which may or may not be observed before the shell is removed. A dirty grayish color, with a more or less yellowish or orange shade in the region of the viscera, is characteristic for parasitized snails. This color, particularly the grayish cast, often persists after the parasites have disappeared from the tissues of the host.

In the spring months, especially March and April, many full-grown snails appear which have a pronounced yellowish to light orange coloration. Upon examination, these rarely yield parasites. Since their gonads are usually intact and in functional condition, they probably were never parasitized to any marked degree and represent individuals that have lost at the most only a light infection. The coloration in such cases may be caused by some other unknown infection or it may be the result of changes that are undergone normally at this season of the year.

Little or no difficulty has been experienced in keeping healthy snails in aquaria provided an abundance of food is present and the aquaria are kept reasonably clean from sludge. In some experiments, supposedly healthy snails were kept in finger bowls, in which the water was frequently changed, in the complete absence of food for periods varying up to sixty days. The criterion for health was appearance. Under this treatment some died early in the experiment, others survived. In many cases those that died early proved to be infected; in all cases those that survived were not infected.

Parasitized snails are difficult to keep for any period of time in an aquarium. They die in a very short time if heavily infected, even in the presence of an abundance of food.

Healthy snails in an aquarium with abundance of food exhibit a degree of activity that may be characterized as moderate. They live in a normal fashion and are able to satisfy their needs without undue exertion. On the other hand, parasitized individuals in many cases have been observed to act differently. In the last stages of parasitism the snail is often immobile and only occasionally bestirs itself to take food. Those that are infected to a lesser degree, moderate to heavy, often feed voraciously and exhibit a more marked degree of activity than do normal ones.

When an attempt is made to remove them, healthy snails maintain their hold on the substratum more tenaciously than do parasitized ones.

DEGREE OF INFECTION

Johnson (1920) reported 86.75 per cent of 400 *Physa occidentalis* examined by him as containing one or more of the various larval stages of *Echinostoma revolutum.* Out of the 400 snails examined by him, 13 harbored mother rediae; 234, daughter rediae and cercariae; 282, cysts in addition to other forms; 95, cysts only; and 13 contained cysts within rediae; 53 were uninfected. *Echinostoma revolutum* apparently exhibits both the monoheteroxene and diheteroxene (Brumpt, 1922) types of life-cycle. The former type of cycle is exemplified in those cases where the agamodistomes are found on the outside of the shell of the intermediate host or within the *same individual* snail in which they had their development; the latter type occurs when encystment takes place in a second individual gastropod or in a *Planaria* (Johnson, 1920). The percentage of infection recorded by Johnson and cited above is a very high one. Hurst (1923) found 16 per cent of 306 individuals of *Lymnaea stagnalis* infected with *Cercaria gunnisoni* and regarded this as a relatively high degree of infection. Many authors have recorded various degrees of infection which range from less than 1 per cent to 100 per cent. Among these, Ssinitzin (1911), who worked with a variety of forms, noted cases varying from 0.4 per cent to 11.0 per cent.

It seems that unusually high percentages of infection are the results of local conditions that are favorable to parasitism; the reverse would be true of the extremely low percentages.

During the course of the present study, 653 specimens of *Physa occidentalis* were examined. Of these, 550 were obtained from the artificial lakes in Golden Gate Park; the remaining 103 from Strawberry Creek on the University campus. Out of the 550 from the park, 352 were infected with larval stages other than cysts as well as with cysts, 108 contained cysts only and 90 were uninfected. This would place the degree of infection at 83.63 per cent. Not a single case of infection with *Echinostoma revolutum* was found in the 103 snails taken from the creek; some were found, however, to contain cercariae other than the one under consideration. These belonged to the *Monostome* and *Xiphidiocercaria* groups. Ducks and geese apparently do not visit this creek. *Echinostoma revolutum* seemed to be the prevailing infection in the snails from the lakes in Golden Gate Park. However, at least three other types were noted. These included monostome, forked-tailed, and stylet cercariae. In a number of cases more than one type was found in the same snail. In such cases, however, either one or the other form predominated in number. Ruszkowski (1922) observed that the number of adult specimens of *Hemistomum alatum* found in the intestine of the fox varied inversely with the number of cestodes and nematodes present.

The degree of infection in individual cases may vary profoundly. Active larvae, when present, may be only few in number or they may practically wholly replace the substance of the hepato-pancreas and the gonad. Faust (1917) recorded that the mass of worms may be twice that of the digestive gland. The cases wherein larvae are few in number are rarer than those in which they are moderate to great in number. Figure 13, plate 17, is a photograph taken from a section of the posterior part of the visceral sac of a heavily infected individual. It will be noted that the gonad has been entirely replaced by parasites and that the epithelium of the hepato-pancreas has been greatly reduced. The mass of parasites occupies nearly all the space. This figure may be contrasted with figure 12, plate 17, which is a photograph of the same region in a normal animal.

In those animals that harbor cysts only, these may be present in enormous numbers and may be found in all parts of the body; they may even be found on the outer surface of the shell. In one case, 466 cysts were counted in the collar of one snail, 572 in that of another. Cases of apparently heavier infection were encountered but no attempts were made to count the cysts. On the other hand, the resting stage of this trematode may be present in such small numbers that infection may be easily overlooked. As stated before, this encysted stage is apparently of no consequence in a pathological sense. Cases of light infections with cysts, no doubt, often pass for normal. Such cases in all probability would not modify the experimental results to any appreciable extent.

PATH OF INFECTION

Fantham (1920) summarized the mode of penetration of the infective stages of the various trematode parasites, particularly the liver fluke, in the case of the sheep and other vertebrates, including man. He said that the miracidium penetrates into an intermediate host which is always a snail or a mussel. Thomas (1883) decided that the entrance of the miracidium of *Fasciola hepatica* into the snail is due to the "softness" of the host, and that it was invoked by a specific attracting substance of a hypothetical nature. He noted that miracidia might enter the foot. As many as a dozen were found to be imbedded in this location. Such miracidia seem to fail to develop. In only one case did Thomas observe a sporocyst in the foot of the host. Sporocysts are found in the pulmonary organ. According to Thomas, from here they "force" their way through the tissues and "wander" to the other organs, especially the liver, and in so doing necessarily do great injury to the host. Cercariae wriggle their way out of the snail by means of their suckers and tails.

Leuckart (1901) observed the miracidia of *Fasciola hepatica* swarm around *Lymnaea truncatulus.* Some of these adhered to the foot, some to the mantle fold, while others entered the breathing pore and finally the lung. Leuckart suggested that the invaders follow the course of the blood and finally lodge in the liver.

As evidence in favor of such a mode of migration as opposed to direct tissue penetration, in the case of the early larval stages, Faust (1920) noted that in the first place the parasites are always located in the connective tissue and the interstices between the tubules of the digestive gland, and that the epithelial cells bordering on the lumina were intact except in the most necrotic cases. In the second place, the food of the parthenitae is derived from the lymph. Agersborg (1924) concluded much the same. The writer has observed the same condition described by both Faust and Agersborg. Even in the earliest stages, beyond the initial act of penetration of the epithelium of the pulmonary organ, migration seems to be by way of the circulatory channels. Evidently the same is largely true of the movements of the cercariae. When the cercariae leave the host they move through the lymph spaces and finally penetrate only the limiting epithelium. The reverse takes place in the cases of cercariae entering to encyst. However, since the cercariae are comparatively large as compared to the normal sizes of the spaces they occupy, much distension does occur during both egress and ingress of the parasites. Where large numbers are present considerable tissue destruction does, as a matter of fact, take place. Taken as a whole, the most harmful stage of the parasite is the parthogenetic phase which inhabits the hepato-pancreas of the snail.

CYCLE OF INFECTION

Undoubtedly the cycle of trematode infection in snails, in regions that have a definite winter season, is a repeated annual one. In the case of the trematodes of water fowls, the eggs would be dropped in the water during the spring migrations and the infective cysts would be ready by the return of the birds during the fall migratory period. A minor cycle might perhaps be introduced under favorable conditions of temperature owing to the fact that some eggs would be dropped in the fall. Infection of the birds could occur in the spring as well as in the fall, however, irrespective of a possible additional cycle in the snail, since the cysts not picked up in the fall might possibly winter through if they happened to be in an intermediate host that lived through the winter. Sewell (1922) described a double rise and fall in the infection rate of certain gastropods in India. The rises occurred from June to August and from December to January. The infections ebbed from March to April and from September to October. He ascribed as one cause of this the migratory habits of birds.

The climate of the San Francisco Bay district of California is comparatively mild and as a result the seasonal cycle of *Echinostoma revolutum* in the snails in the lakes of Golden Gate Park is not markedly regular. A definite annual cycle does exist, however, in which infection of the young snails takes place in the spring and cysts are ready to infect the waterfowls in the fall. This is borne out by the fact that mature cercariae are much more abundant from July to November than during the other months of the year. During January and February no cercariae are to be found, although rediae are abundant. As suggested by Johnson (1920), there is much overlapping of the stages. This is partly, at least, due to the fact that some water birds are to be found on the lakes in Golden Gate Park throughout the year. A great majority of the ducks and geese, however, raise their young farther north

With regard to the cycle of infection within the individual snail, the writer is not prepared to be wholly in agreement with that given by Agersborg (1924). This latter author describes four infection stages as follows (p. 390):

> (1) The parasite invades the host. The tissues shrink and become more and more difficult to handle for cytological purposes. The tissues become friable. (2) The tissues secrete an intercellular granular substance which becomes present everywhere in the host. It is not present in the parasite. (3) During prolonged heavy infection the tissues disintegrate. The epithelium of the liver may be reduced from tall columnar to squamous. The host may die. (4) If the host does not die, then there follows after the third stage a gradual return to the normal. The parasites also decrease in number within the host.

The evidence obtained by the writer seems to indicate a cycle of a somewhat different kind. This cycle may be briefly stated thus: (1) infection; (2) multiplication of the parasites; (3) tissue degeneration and tissue proliferation on the part of the host; (4) death of the host, or emigration of the parasites and death or recovery of the host.

COMPARISONS OF HEALTHY AND PARASITIZED PHYSA OCCIDENTALIS

This section deals directly with the structural and functional changes in *Physa occidentalis* that are incident upon infection with the larvae of *Echinostoma revolutum.* For convenience it is divided into three more or less distinct subdivisions; namely, (1) histological and cytological changes, (2) variable factors influencing metabolism, and (3) experimental studies on metabolism and metabolic products. The first of these three subdivisions deals only with prepared material; the last two with the living animal and the products of its activity.

HISTOLOGICAL AND CYTOLOGICAL CHANGES

For the study of any given point both healthy and parasitized individuals were used. These were subjected to identical procedures in the process of preparation for examination. In all cases direct comparisons have been made between the conditions that prevail in both health and parasitism. Even though the snail used in this study has a very high percentage of infections, many individuals taken from the natural environment were found, upon being sectioned, to be absolutely free of trematode larvae.

Agersborg (1924, p. 374) said,

> Since all the specimens I examined were infected at the time of examination, it was impossible to ascertain from them the exact nature of the normal tissues. But judging from many cases with an early or mild infection, I believe that upon infection the tissues become less responsive to ordinary stains. Infected specimens are difficult to fix properly. The tissues have a tendency to crumble before the edge of the knife. None of these difficulties were met with in the uninfected material which was raised in the laboratory. The normal tissue has less tendency to shrink during the process of preparation.

According to this statement, the healthy specimens used by Agersborg were laboratory-grown and probably far from mature when subjected to fixation for microscopic preparations. The present writer believes that this was a possible source of error, as will be pointed out later. The experiences of Agersborg with regard to the relative degree of responsiveness of the tissues of normal and parasitized snails to fixing agents and stains have been encountered in this work. The writer believes, however, that proper fixation is the most important step and that it may be obtained by using the fixing solution hot (60° C.). Excellent results were most certainly obtained with both healthy and heavily infected material by this method and no difficulties were met with in subsequent sectioning and staining in material thus fixed.

STRUCTURAL AND FUNCTIONAL CHANGES IN THE FOOT

A marked degree of infection produces profound changes in the tissues of the snail. The changes in the foot, although not so evident as those in the organs of the visceral sac, are nevertheless characteristic.

The foot of *Physa* is a muscular locomotor organ. It is composed largely of loose connective tissue closely interwoven with the well developed and numerous muscle fibers. Many lymph spaces are present throughout the organ. In these, and in the loose connective tissue, are often observed many wandering cells or amoebocytes. The so-called *cellules de Leÿdig* occur in the musculature of *Physa occidentalis*, often in very marked degree. As pointed out before (p. 328) these cells apparently serve a triple function. The foot as a whole is covered with a columnar epithelium that is underlaid with numerous well developed slime glands. This epithelium usually carries an abundance of pigment granules. Pigment cells are present throughout the musculature; the pigment itself may be both intracellular and extracellular.

Macroscopically, in cases of heavy infection, the foot appears to be much shrunken in the living animal. Its cross-section, in proportion to its length, is less than that in a healthy individual with the same size of shell. In internal structure the foot of the parasitized individual is quite different from the normal one. The muscular tissue is much reduced in amount and the lacunae are more prominent. Figures 17 and 18, plate 17, show the condition of the musculature of the foot of a healthy and a parasitized snail respectively. The conditions illustrated are extremes but are uniformly present in such cases. In cases of heavy infection both the amoebocytes and the Leydig cells are reduced in number in the foot. The amount of glycogen, as revealed by Best's carmine and by the iodine test, is greatly decreased, or even practically absent, in the muscular tissue and Leydig cells of the parasitized snail.

According to Agersborg (1924) miracidia, rediae, and cercariae, in all stages of development, inhabit the foot as well as all the other tissues of the body. His figure 1, plate 12, shows a parasite which he interprets as a cercaria below the ectoderm of the foot. From the appearance of the figure this is very evidently an encysted cercaria, or cyst. One gathers the impression from the paper cited that the larvae are capable of undergoing development in the foot. This is contrary to the opinion of Faust (1920) and the present writer has never seen any stage other than the cyst actually present in the tissues of the ventral locomotor organ. It is known, however, that miracidia will enter the foot (Thomas, 1883; Leuckart, 1901; Faust, 1920), but if they do so they fail to go through their cycle. Thomas (1883) recorded only one case of a sporocyst present in the foot.

One of the most characteristic features of the foot of the normal full-grown *Physa* is the presence of numerous, minute, spherical pigment granules in the epithelium and throughout the underlying tissues themselves. These granules are present not only in the foot, but in the mantle and in the tissues of the entire body in general. They are brownish to deep black in color. Haematoxylin preparations show them to the best advantage. They are, however, preserved by a variety of fixatives—both with and without acetic acid. Unstained, fixed material clearly shows them; unfixed smears contain them.

These granules, as said before, occur both intracellularly and extracellularly. It is possible that they are nuclear in origin. This point is discussed later.

In cases of heavy infection these pigment granules are greatly reduced in quantity; in light infection they are little changed from the condition of abundance found in health. Very young healthy animals do not possess them in a well developed state. It is only in mature individuals that they reach their full development. Figures 14 and 15, plate 17, show the condition of these granules in a healthy snail. Figure 16, plate 17, shows the condition in the same location in a heavily parasitized one. All gradations between these two extremes may be found. There is little room for doubt that parasitism has a profound quantitative effect upon these elements. The effect seems to be expressed as an inhibition of production of new granules in the inner tissues and a gradual excretion of them through the epidermis.

Agersborg (1924) described granules which he interpreted as ''cytological secretions'' and an ''antidote'' elaborated on the part of the host. He described these as being present in abundance in light infections and progressively decreasing in heavy infections. According to him, they are entirely absent in healthy specimens.

The writer has found no granules in *Physa occidentalis* that agree in all respects with those described by Agersborg. Rather, he has found only the normal pigment that behaves as stated. Both authors agree, however, that this pigment is progressively reduced from moderate infection to heavy infection. The fact that Agersborg did not find the granules in healthy material may be explained by the fact that he used immature specimens.

STRUCTURAL AND FUNCTIONAL CHANGES IN THE HEPATO-PANCREAS

The hepato-pancreas of *Physa occidentalis,* in common with that of other pulmonates, is a compound racemose gland. It arises from two embryological outpocketings from the mid-intestine and is composed of a glandular epithelium which lines the tubules. These tubules are bound together by a network of interstitial cells and the organ as a whole is encased in an epithelial sac, the tunica propria. The cells which compose the secretory epithelium have been the subject of numerous researches, summarized in a previous section.

Contrary to the European workers and in agreement with Faust (1920) the writer is able to recognize two different types of cells in this epithelium. The first of these is the so-called lime cell of Barfurth (1883*a*) which Faust describes as being rhomboidal or tetragonal in shape and possessing large spherical nuclei. The second and more numerous type of cell, corresponding to the ferment cells and liver cells of Barfurth (1883*a*), is designated by Faust as being irregularly palisade in character and having small oval nuclei.

The lime cells of *Physa occidentalis* are triangular to tetragonal in shape and are almost invariably basal in position; that is, they are applied to the basement membrane and their free ends rarely reach the lumen of the duct. The nuclei of these cells are characteristically large and spherical with one large karyosome variably located and with numerous scattered chromatin granules. In this snail no lime cells have been seen that possessed a polymorphonuclear form as described by the earlier European workers in various gastropods. The exact nature of these cells remains in doubt even at the present time. Barfurth (1883*a*), as well as many subsequent authors, regarded them as storage cells for tri-calcium phosphate. Faust (1920), as has been stated, considered these cells as protein-storage cells on the basis that their reaction to dyes suggested a complex mucoid. The writer is of the opinion that both these interpretations are more or less correct. Following fixation in absolute alcohol, positive results may be secured with Roehl's method for calcium deposits as outlined by Mallory and Wright (1921) (pl. 18, fig. 29). The granules of these cells probably consist of calcium salts in an organic matrix of a mucoid nature. Faust's (1920) description of their behavior in pathological cells, however, is strongly in favor of his interpretation; but this behavior is not inconsistent with the presence of calcium salts. He described these granules as degenerating during the course of parasitism and accumulating as dense agglomerates between the nucleus and the free end of the cell. Later they become worn away and dispersed throughout the cell, adding to the excretory wastes. The exact nature of these granules will perhaps only be settled by dissecting them out with a micropipette and subjecting them to careful microchemical tests.

The second type of cell present in the hepato-pancreas of *Physa occidentalis* is roughly palisade in character, with a small nucleus that varies from spherical to elongate oval in shape. As in the case of the lime cell, the nucleus of this second type of cell characteristically possesses one irregularly disposed karyosome and numerous chromatin granules. These cells vary considerably in size and in appearance. They vary in appearance according to their physiological state and the degree to which they are performing their various functions. The functions of absorption, storage, secretion, and excretion are apparently the office of these cells. Any one of them is capable of performing all these duties; but, as a matter of fact, in many cases one or the other may predominate in any one given cell. Such a predominance may be only transitory, but there is no way to prove this latter point. The normal cell of this type usually contains either a large secretion vacuole, or an excretion vacuole, or, in some cases, both. These vacuoles vary considerably in size and their natures are revealed by the staining reactions of their contents. They also vary in number. Fat and glycogen are present as storage products in these cells in varying amounts. Figure 8, plate 16, shows the general nature of the two types of cells of the hepato-pancreatic epithelium of *Physa occidentalis*. In this figure the fat and glycogen are not shown since the preparation from which it was made was not stained to bring out these substances.

The hepato-pancreas is the seat of some of the most profound changes produced by fluke infection. It is here that the parasites undergo their parthenogenetic development with its attendant enormous increase in numbers. They are lodged in the intertubular sinuses and in this location they are in the path of the normal food distribution of the host. The parasites require food for their growth and multiplication. This must be supplied by the host. Faust (1920, p. 81) said,

> This heavy drain on the food supply of the host is easily demonstrated. Under normal feeding conditions snails which are not infected give a heavy test for glycogen, fats and proteins throughout all of the cells of the epithelium of the tubules. Most of the glycogen and fats are in the liver cells, while the protein storage cells give a heavy test for protein materials. On the other hand, heavily infected tissues give at most only a very scanty test for foodtsuffs in the epithelium, a fairly heavy test in the intertubular sinuses, and a decidedly heavy test in the parasites.

The writer is able, from his own studies, to agree with this statement with the one exception of the relations of the fat. As is pointed out later, this substance is apparently increased in parasitism.

The general appearance of sections of the viscera of heavily infected snails is enough to tell that the condition of the hepato-pancreatic tubules that remain is far from normal. They have a disintegrated aspect in many places; necrotic areas in which cell boundaries are lost in a mass of cell debris are common (pl. 18, fig. 28). The nuclei of these cells seem to resist disintegration to the last. On the other hand, in more moderate infections, one is surprised at the degree to which the epithelium can retain its normal aspect.

A common reaction that may be noted on the part of the nuclei of the cells of the hepato-pancreatic epithelium is that there is a tendency for them to migrate away from the base of the cell toward the lumen of the tubule in cases of heavy parasitism. This is especially noticeable in portions of the epithelium that are immediately underlaid by parasites in the intertubular connective tissue (pl. 17, fig. 19). In the normal cells the nuclei lie basal in all cases. Agersborg (1924) interpreted this as a migration of the nuclei toward the food supply in the lumen since the parasites abstract food from the basal parts of the cells. The extrusion of chromidia, as described by Faust, has been noted in only a few instances.

In many cases the parasites are so numerous in the sinuses that the lumina of the tubules are closed as a result of the pressure brought to bear upon them. The basement membrane in many places all but disappears.

The process of degeneration of the lime cells in *Physa occidentalis* seems to be somewhat similar to that pictured by Faust (1920). There is a breaking down of the material of the granules to form a dense agglomeration which later becomes dispersed throughout the cell (pl. 16, fig. 11). In some of these cells, on the other hand, the process seems to be a more gradual dissolution of the granules and their complete dispersal, with a dense basophilic reaction of the cell as a whole as the end result. On the whole, however, these lime cells are more refractory than the other type of cells. They often retain their characteristic granules when the other cells have degenerated to the point of necrosis.

The so-called liver cells, in health, present two different types of vacuoles (pl. 16, fig. 8). These are the secretion vacuoles and the excretory vacuoles. The former contain granules that are either basophilic or remain unstained; the latter are invariably acidophilic. Up to the point where actual disintegration begins to set in, there does not seem to be much change in the nature of the secretory products. After this point they become eosinophilic and add to the wastes that go to make up the final necrotic mass. The excretory vacuoles together with their contained masses are greatly augmented in parasitism (pl. 17, fig. 20). Even in health, however, these vacuoles are very evident and may contain small concretions of katabolic products. The added wastes of the parasites are evidently partly passed through the epithelium of the hepato-pancreas since the excretory vacuoles show a marked increase in eosinophilic contents. Beyond a certain point the contents of these vacuoles seem to have a lytic effect upon the cell, for they lose their clear-cut outlines, and the next step is the more or less complete disintegration of the cell. The final necrotic mass is loaded with eosinophilic material. The reaction of the normal epithelium seems to be basophilic; that of the unhealthy epithelium is decidedly acidophilic when the degree of infection is heavy. In earlier stages, however, as noted by Faust (1920), only the free ends of the cells are acidophilic, indicating a tendency on the part of the host to counteract the acidity by rapidly filtering the wastes into the lumen.

The amount of glycogen in the cells of the epithelium is all but entirely reduced in parasitism; in heavy infection it is entirely lacking so far as its presence is capable of being revealed by iodine or Best's carmine. The fat, however, seems to be influenced in a different way. In many preparations, more of this substance is found in the epithelium of the hepato-pancreas of infected snails than in that of healthy ones. Since this condition seems to be associated with a reduction in the amount of mitochondria present, the fat is probably not stored directly after absorption but represents a truly pathological condition, perhaps comparable to fatty degeneration in the vertebrate liver. Figures 2 and 9, plate 16, show the relative amounts of fat present in the two epithelia.

The so-called Golgi bodies, or paranuclear bodies of Agersborg, which are probably concerned with the secretory functions of the cells, are located basally in close proximity to the nucleus. Some often lie between the nucleus and the basement membrane. They are variable in number and in size. In structure, they are decidedly granular. Those distal to the nucleus are often found in a vacuole—the secretion vacuole. Their behavior in parasitism is not suggestive. Prior to the initiation of cell disintegration, they seem to maintain their integrity. Figure 27, plate 18, shows these bodies in the cells of the epithelium of the hepato-pancreas of a parasitized snail.

STRUCTURAL AND FUNCTIONAL CHANGES IN THE KIDNEY

The emunctorium, or kidney, of *Physa occidentalis* does not differ in any essential detail from those described by the various authors as occurring in other gastropods (Schneider, 1902; Meisenheimer, 1912; *et al.*). This organ consists largely of more or less cubical cells affixed to a thin structureless basement membrane that is underlaid with connective tissue. The whole is thrown into numerous folds or lamellae and surrounds a spacious cavity. The connective tissue is often all but obliterated between the folds except for a bulb-like blind end, in lamellae the sides of which closely approach each other (pl. 16, fig. 3). This tissue, however, is the seat of the transfer of waste products from the blood and lymph to the excreting cells of the renal epithelium. It contains sinuses and lacunae that are in morphological communication with the circulatory channels. Amoebocytes may often be observed in these spaces and in the narrow interstices in the lamellae themselves (pl. 19, fig. 32).

Strohl (1914) analyzed three possible ways by which the waste products could reach the nephridial cells. In the first place, they may enter these cells directly from the blood in a soluble form. Secondly, they may enter in the form of solid particles. The third, way would be for these materials to be taken up by the wandering cells and delivered to the cells of the excretory epithelium. It is probable that all three of these processes occur in *Physa occidentalis*. Many extracellular granules are often seen in the connective tissue of the lamellae. Some of these are identical in appearance and behavior with the granules seen in the vacuoles of the nephridial cells; some are identical with the pigment granules previously described. Many amoebocytes may sometimes be encountered in the lacunae adjacent to the bases of the nephridial cells and even in contact with these cells themselves. These amoebocytes frequently are observed to carry brownish granules similar to those seen in the excreting vacuoles. Meyer (1925) recorded a similar conclusion but said (p. 25), "The nephridial cells of the areas invaded by the amoebocytes are particularly rich in excreta, in comparison to other zones on the same lamella." This latter behavior has not been observed by the writer to any marked extent in the nephridium of the snail he studied. The transfer of granules from the amoebocytes to the excreting cells of the renal epithelium, as a method of excretion, may be only secondary in importance in *Physa occidentalis*.

Both uric acid and urea are present in the kidney of *Physa occidentalis*. Positive reactions were obtained for the former substance with both the murexid test and the phosphotungstic acid reagent of Folin (1923). Urea was shown by means of the sodium hypobromite reagent of the same author. For each test several kidneys were dissected as free as possible of the other tissues and dried. The dried material was used for the murexid test, extracts were prepared for the other two tests.

The renal epithelium of the healthy snail presents a very different picture from that of the parasitized one. In the former case the cells are vacuolated in a strikingly uniform manner. The vacuoles are for the most part clear-cut and contain varying amounts of granules and concretions. These are greenish to yellow or brown in color. They do not take a basic stain.

Ordinarily the renal epithelium is only one cell in thickness but places may be found, especially at the tips of the lamellae, where it is sometimes more than one cell in depth. This condition is exceptional, however, and is not to be confused with the 'foam-like' appearance of the accumulated excretory vacuoles (pl. 19, fig. 32). Figure 31, plate 19, shows the general nature of this organ in a healthy animal.

Faust (1920) expressed the opinion that the effect of the parasite on the excretory process of the mollusk is twofold. The host is required to dispose of the parasite's wastes and at the same time of increasing amounts of its own. Part of this is attempted by the hepato-pancreas; part by the kidney.

The renal epithelium of the heavily parasitized *Physa* invariably shows larger and more dense agglomerations of excretory products than that of the healthy animal (pl. 16, figs. 1 and 3). These agglomerations are heavily stained by the silver-hydroquinone process. The case illustrated in figure 1 is an extreme one. The vacuoles are not so clear-cut; their margins are ragged. In some places, a condition suggesting necrosis occurs. Figure 30, plate 19, makes clear the appearance of the excretory lamellae of a very heavily infected individual.

The nuclei of the renal cells are basal in position and usually spherical in shape. They each possess, as a rule, but one karyosome and a variable number of chromatin granules. The karyosomes in the renal cells in instances of marked infection have a disintegrated aspect in that they are decidedly more granular in structure than those found in healthy cells (pl. 16, figs. 4 and 5).

No evidence whatever has been found for a method of excretion other than the merokrinous, and, in some cases, the defective elimination of particles from the vacuoles. The holokrinous type of excretion apparently does not occur in the nephridium of the pulmonate under consideration.

STRUCTURAL AND FUNCTIONAL CHANGES IN THE ALIMENTARY CANAL

Aside from the glands, of which the hepato-pancreas is the main one, the alimentary tube of *Physa occidentalis* is made up of the buccal cavity, oesophagus, gizzard, stomach, and intestine. This is the same fundamental plan of structure found in the other basommatophorous pulmonates and in the gastropods as a class as described by Pelseneer (1906). In the floor of the buccal cavity is found the radular pouch. The dorsal wall bears the chitinous upper jaw. The gizzard is thick-walled and muscular; the stomach is thin-walled. Cilia are found in the posterior part of the buccal cavity, in the oesophagus, the stomach, and the intestine.

Throughout the alimentary tube the epithelium is columnar. In the buccal cavity the cells are almost cubical. Those of the gizzard are narrow and very much elongated. The stomach and intestine are characterized by cells rather intermediate between the other two types. All these cells have nuclei that are oval in shape, in the clear sap of which may be distinguished one or more karyosomes as well as additional chromatin granules.

In heavy infections with trematode larvae several changes of interest may be noted. In the buccal epithelium, posterior to the chitinous upper jaw, in material prepared by the Da Fano modification of the Cajal technique, it may be observed that the spherical Golgi bodies are increased in size and number (pl. 16, figs. 6 and 7). This effect is just the reverse of that which may be noted in the mid-intestine. In this latter organ, these bodies seem to be all but absent in parasitized snails (pl. 18, fig. 21). The significance of these bodies in these two locations is doubtful. Their behavior, however, is suggestive of an attempt at a compensatory action on the part of those in the buccal epithelium. The small spherical mitochondria in the same cells are apparently little changed in quantity.

The nuclei of the alimentary epithelium as a whole seem to survive even heavy cases of infection with little noticeable change. In many cases, however, the karyosomes disappear, but the chromatin material as a whole is otherwise dispersed very much as in the normal cell (pl. 18, figs. 23 and 24).

The nuclear caps in the intestinal epithelium of the snail described by Agersborg (1924) are also to be found in the fore-gut of *Physa occidentalis,* in the stomach, and in the gizzard. They are well demonstrated by the Da Fano method and consist of granular masses between the nucleus and the lumen. From them strands of granules pass toward and even into the lumen (pl. 18, figs. 25 and 26). In some instances the individual granules of these strands cannot be made out and a fibrillar appearance is evident. Undoubtedly these bodies are secretory in function and of a nature allied to that ascribed to the so-called Golgi material. If this material is in reality secretory in function its integrity would be vital to the snail in disease as well as in health. From this standpoint, one would expect little or no change in the amount and distribution of this material from health to the parasitized condition. This, in fact, seems to be the case (pl. 18, figs. 25 and 26) in cells that maintain their integrity. However, the stainability of the granules in disease is much less than in health.

STRUCTURAL AND FUNCTIONAL CHANGES IN THE CONNECTIVE TISSUE

The connective tissue is the seat of many far-reaching effects that are produced in the snail by infection with trematode larvae. It is in this tissue that the parthenogenetic generations are harbored in the intertubular spaces in the hepato-pancreas. Contact with the parasite and its products is here of the most intimate nature. This tissue is the first to be affected and the first to respond. Hypertrophy takes place with the subsequent production of fibrous capsules about the invaders. In late stages of parasitism there is considerable atrophy of the connective tissue. This is not strictly confined to the connective tissue in direct contact with the larval flukes but may be seen in other parts of the body—even in the foot.

When the parasites have developed to the stage where active cercariae are present, there is much lysis of the connective tissue which is probably caused by the secretions of the cephalic glands of these animals. The nuclei of these connective tissue cells appear, however, to resist digestion and may be found free in a structureless mass of débris (pl. 16, fig. 10). Agersborg (1924) described a "syncytium" underlying the basement membrane of the hepatopancreatic epithelium that probably represents a case of this kind.

In healthy snails numerous amoebocytes are found in the lacunae of the connective tissue in all parts of the body. These are apparently much reduced in number during the course of the extreme diseased condition (pl. 19, figs. 33 and 34).

According to Faust (1920), upon the increase of parasites in the connective tissue a condition of stasis is produced. This is conducive to a slowing up of the circulatory rate and the accumulation of wastes which greatly augment the abnormal reaction of the connective tissue as well as the adjacent glandular tissue.

STRUCTURAL AND FUNCTIONAL CHANGES IN THE GONAD

Not much can be written at present of the changes in the gonad, that are attendant upon the parasitized condition of the animal. In severely diseased snails this organ has entirely disappeared. Its place is taken by a solid mass of parasites. This fact has been previously noted by a number of authors, among them Sewell (1922). In light infections, and in infections wherein cysts only are present in moderate numbers, this organ appears to suffer no harm. Where the number of cysts is great, considerable disappearance of reproductive tissue through pressure atrophy ensues. In early infections with large numbers of cysts development may be much retarded.

If the infection is acquired after the hermaphrodite gland has developed, this organ will probably continue to function unless the infection progresses so far as to cause degeneration. Another possibility is that an infection with the reproducing parthenogenetic parasites, acquired early, may entirely inhibit the functional development of the gland.

That parasitism has a profound influence on the sexual development of the individual, may be seen in many groups of animals—and even in man himself. A classical instance is that of *Sacculina* parasitic on Brachyura (Calman, 1909). In this case, complete castration may occur. The male Brachyuran often shows a complete reversal of its secondary sexual characters. In man severe hookworm infection often retards the development of the sex organs to a very marked degree (Dock and Bass, 1910).

VARIABLE FACTORS INFLUENCING METABOLISM

Several variable factors must be taken into account before it is possible to arrive at a critical conclusion as to the effects of parasitism upon the metabolism of the host. In the present study on the metabolism of *Physa occidentalis,* in health and in infection, three major factors which influence the metabolic rate were considered. These were the effect of weight or age, the effect of degree of locomotor activity, and the effect of the physiological state. Such factors as time and temperature, except in special cases, were not taken into mathematical account since the experiments were carried out under approximately uniform conditions with respect to them.

EFFECT OF WEIGHT OR AGE

In his work on the metabolism of *Planaria dorotocephala,* Child (1919) regarded size as a criterion of age in the animals. As a general rule, this same criterion applies to *Physa occidentalis.* However, the relative sizes of two given individuals of the latter species are difficult to judge accurately. As a consequence of this, weight was used as the measure of age. No definite standardizations of certain weights to certain ages were attempted as such were deemed unnecessary for the purposes of these experiments.

Other factors being equal, that age and weight, within limits, are closely correlated in *Physa occidentalis,* is a reasonable conclusion. However, growth in these animals does not go on indefinitely. There is a limit to the size that may usually be reached and it is the exceptional individual that exceeds this. When the usual adult size is attained there is perhaps no longer so direct a relation between age and weight. Most individuals of the species of snail collected by the writer averaged less than ten millimeters in length of shell. A few measured as much as fourteen millimeters. One of the latter (14 mm.) weighed 0.2830 gram, another (11 mm.) weighed 0.2289 gram. Since no snails that weighed more than 0.1750 gram were used in these experiments on metabolism, it is believed that they represented a selected group of individuals in active growth and approaching full maturity of size.

In experiments of the kind necessary to arrive at the nature of the effect of weight or age on metabolism, it is obviously impossible so to arrange them that uniform conditions will be assured from the outset. Rather, it is necessary to conduct a large number of individual experiments and from them select those that fulfil the requirements. To determine the part played by weight, the factors of locomotor activity and the duration of the test in each individual case should be constant. The time is easily enough controlled but this cannot be said of the locomotor activity of the animal being used in the experiments.

Out of over three hundred separate tests of carbon dioxide production and oxygen consumption by the methods described, only fifteen cases yielded results that could be used to throw light upon the particular point in question. The rest had to be rejected on account of nonconformity in the matter of average locomotor activity. The two tables below show the results of the fifteen valid cases. Table 1 records the data for eight cases wherein the weights of the snails are approximately equal. The degree of activity (expressed by 3.3, a term to be explained in the next section), as well as the time, is the same in each case.

TABLE 1

CARBON DIOXIDE PRODUCTION BY SNAILS OF UNIFORM WEIGHT AND ACTIVITY

No.	Weight in grams	Average activity	pH change	Time in hours	Temperature
1	0.0621	3.3	0.4−	2.5	Room temperature in all cases. Varied but slightly from 20° C.
2	0.0319	3.3	0.4−	2.5	
3	0.0650	3 3	0.4	2.5	
4	0.0643	3.3	0.4−	2.5	
5	0 0642	3 3	0.4−	2.5	
6	0.0632	3 3	0.4+	2.5	
7	0.0631	3.3	0.4+	2 5	
8	0 0648	3.3	0.4−	2.5	

In all these cases the production of carbon dioxide, as measured by the indicator (Phenolsulphonphthalein), varied but little around a change of 0.4 (i.e., from 8.2 to 7.8). Since a standard set of buffer solutions was made up that covered the range in steps of 0.2 each (e.g., 6.8, 7.0, 7.2, etc.), it was necessary to estimate the intervening points (e.g., 6.9, 7.1, etc.). The color perception of the writer is sufficiently acute to make such estimates accurately. Cases occurred, however, in which the end point did not seem to lie exactly on a given 0.1. In such instances a plus or minus estimation was recorded.

The eight snails for which data are given in table 1 were selected from a large number of experiments. In none of these experiments did the control tube, which was in all cases run at the same time, show any change in the hydrogen-ion concentration.

The writer believes that the hydrogen-ion method for measuring carbon dioxide production furnishes a ready method for such measurements wherein the results desired are for comparative purposes and not for exact quantities of this substance. That the change in color of the indicator is produced by carbon dioxide is evidenced by the fact that the original pH could be restored to all tubes, after removing the snail, by gently heating the contents. The chance that any other volatile acid would be responsible for the change of color is negligible. Child (1919) expressed the same view.

Table 2 lists seven snails of varying weights but exhibiting identical degrees of locomotor activity, that were each tested for two and one-half hour periods.

TABLE 2

CARBON DIOXIDE PRODUCTION BY SNAILS OF UNEQUAL WEIGHT BUT UNIFORM ACTIVITY

No.	Weight in grams	Average activity	pH change	Time in hours	Temperature
9	0.1312	3.0	0.7+	2.5	Room temperature in all cases. Varied but slightly from 20° C.
10	0.1151	3.0	0.6+	2.5	
11	0.1077	3.0	0.6−	2.5	
12	0.1030	3.0	0.5+	2.5	
13	0.0942	3.0	0.4+	2.5	
14	0.0536	3.0	0.3+	2.5	
15	0.0307	3.0	0.2	2.5	

This table shows two things. It shows, in the first place, that the production of carbon dioxide is roughly directly proportionate to the weight of the snail. This, indeed, might naturally be expected. In the second place, the lighter, or younger, snails produce relatively more carbon dioxide in the unit time than the older ones. The numerical expression of the weight of snail 15, the lightest and youngest one, has the relation $\frac{0.0307}{0.2}$ or 0.153 : 1 to the numerical expression of the pH change. That for snail 9, the heaviest and oldest, has the relation $\frac{0.1312}{0.7}$ or 0.188 : 1. Since these derived decimals show the carbon dioxide production in proportion to weight in each case, it is to be seen that this proportion is relatively greater in the case of the lighter snail. The true comparison between the two individuals is obtained by reversing the decimals so that the proportion becomes 0.188 : 0.153 for thé light and heavy snails respectively. Child (1919, p. 234) recorded a similar phenomenon in *Planaria dorotocephala* in that physiologically younger animals metabolized at a more rapid rate than older ones. His results would give a relation of 10 : 13 in the case of old and young animals respectively, as compared to the 0.153 : 0.188 or 10 : 12 relationship in the same order for the two snails above-mentioned.

The results recorded above for carbon dioxide production as related to the weight of the snail are supported by the data obtained on oxygen consumption in the Thunberg-Winterstein apparatus.

TABLE 3

OXYGEN CONSUMPTION BY SNAILS OF UNEQUAL WEIGHT BUT UNIFORM ACTIVITY

No.	Weight in grams	Average activity	cc. O_2*	Time in hours	Temperature
16	0.030	4.0	0.0158	4	Room temperature in all cases.
17	0.073	4.0	0.0328	4	Varied but slightly from 20° C.
18	0.118	4.0	0.0534	4	

* 0° C., 760 mm. of mercury.

This table, although recording limited data, shows that oxygen consumption varies directly with the weight but is relatively greater in the lighter individual. The proportion of the numerical expression of the weight of snail 16 to the numerical expression of its oxygen consumption is $\frac{0.030}{0.0158}$ or 1.9 : 1.0. That of snail 18 becomes $\frac{0.118}{0.0534}$ or 2.2 : 1.0. By reversing these decimals the relationship becomes 2.2 : 1.9 for the light and heavy snails respectively. Concidentally, this is approximately the same as the proportion found in the case of carbon dioxide production as set forth above.

EFFECT OF DEGREE OF ACTIVITY

Snails that are undergoing tests of their metabolic rates, either by the colorimetric or the respirometer methods, exhibit varying degrees of locomotor activity. This activity, as will be subsequently shown, has a marked effect on the respiratory exchanges. Obviously, then, any statement of general results that would fail to take locomotor activity into account would be inaccurate.

The rate of increase in carbon dioxide production or of oxygen consumption in *Physa occidentalis* (a poikilothermal animal), as related to increases in muscular exertion, is apparently not so profound as it is in homoiothermal animals. At the most, the writer has found that the metabolism of the fully active snail is approximately double that of the resting one. By resting it is not implied that a state of hibernation is to be understood, but rather the normal, well-fed animal at rest. In man (a homoiothermal animal) according to Starling (1920) the rate of gaseous exchange may be increased as much as ten to fourteen-fold in passing from a state of complete rest to one of exhaustion as the result of severe work. However, the degree of activity of the snail that is regarded as the maximum in these experiments probably does not lead to anything near a state of exhaustion even when continued for extended periods.

In order to record the degrees of locomotor activity of the specimens used, an arbitrary system of numbers was devised. The different degrees of activity and their designations are listed in table 4.

TABLE 4

DEGREES OF ACTIVITY

Designation	Activity	Remarks
1	Quiescent	Animal completely retracted into shell.
2	Inactive	Foot applied to substratum. Tentacles withdrawn. No locomotion.
3	Feeble locomotion	Tentacles out. Occasional exploratory movements. Translocation less than 1 cm. in 10 minutes.
4	Moderate locomotion	Intermittent or slow to moderate movement.
5	Active locomotion	Constant locomotion in normal manner.

In the respirometer only one snail at a time could be observed. Any number, ordinarily twenty, could be observed when they were in individual tubes for colorimetric determination of carbon dioxide production. As a usual rule six observations were made on the activity of each snail at intervals which varied with the duration of the experiment. An average of six readings was recorded as the final result.

Out of the same group of experiments referred to in connection with tables 1–3, a number of individuals were selected, the data on which were applicable to the question of effect of muscular activity. In these cases snails of nearly the same weight were chosen.

TABLE 5

OXYGEN CONSUMPTION BY SNAILS OF SIMILAR WEIGHT BUT UNEQUAL ACTIVITY

No.	Weight in grams	Average activity	cc. O_2*	Time in hours	Temperature
19	0.0872	2.6	0.0191	4	Varied slightly, about 20° C.
20	0.0868	3.4	0.0266	4	
21	0.0829	3.6	0.0269	4	
22	0.0879	3.8	0.0283	4	
23	0.0865	3.8	0.0294	4	

* 0° C., 760 mm. of mercury.

TABLE 6

CARBON DIOXIDE PRODUCTION BY SNAILS OF SIMILAR WEIGHT BUT UNEQUAL ACTIVITY

No.	Weight in grams	Average activity	pH change	Time in hours	Temperature
24	0.0690	2.0	0.4–	2.5	Varied slightly, about 20° C.
25	0.0617	2.0	0.4–	2.5	
26	0.0639	2.0	0 4	2.5	
27	0.0647	2.0	0.4–	2.5	
28	0.0652	2.5	0.4	2.5	
29	0.0649	2.7	0.4+	2.5	
30	0.0623	3.0	0.4+	2.5	
31	0.0642	3.3	0.5	2.5	
32	0.0677	3.8	0.5+	2.5	
33	0.0620	4.0	0.6	2.5	
34	0.0683	4.2	0.6+	2.5	
25	0.0661	5.0	0.8	2.5	

These tables show in a graphic manner that the relation here, as in the case of weight or age, is a direct one. It is rather striking that the activity designations of snails 19 and 23 bear the relation of approximately 2:3 and that their respective consumptions of oxygen bear closely the same relation. The intervening three cases do not fit into the scheme quite so nicely but are not far enough off to upset it. In table 6 the first seven individuals show approximately the same carbon dioxide production, namely, an amount corresponding to a pH change of nearly 0.4. It will be noted, however, that there is a slight increase in ascending the range of activity covered by these seven cases when the plus and minus notations are taken into account. The last six individuals, ranging from 3 to 5 in activity, show a rapid increase in the carbon dioxide production. From the data presented, it may be concluded that in passing from an average activity as expressed by approximately 2.5 (midway between 2 and 3, the range of activity that produced a P_H change of 0.4) to one expressed by 5, the amount of carbon dioxide eliminated is doubled. In the experiments that are recorded in the following section on comparative experimental data, no snails were used the average activity of which dropped below 2.5.

Table 7 gives the results of an experiment in which one snail was subjected to six hours' observation in the respirometer. A total of thirty-six observations were made on its degree of activity at ten-minute intervals. The reading of the movement of the oil drop in the capillary tube connecting the two chambers was recorded each hour. Only actual readings on the millimeter scale are given in this table.

TABLE 7

OXYGEN CONSUMPTION OF A SINGLE SNAIL

Time	Reading	Activity at 10-minute intervals	Average activity	Change in reading
9:40 a.m.	73.8	5, 5, 5, 5, 5, 5	5.0	
10:40	79.0	5, 2, 2, 2, 2, 2	2.5	5.2
11:40	81.0	2, 2, 2, 3, 3, 2	2.3	2.0
12:40 p.m.	83.3	2, 3, 2, 2, 2, 2	2.1	2.3
1:40	85.4	2, 4, 3, 3, 4, 3	3.1	2.1
2:40	88.0	2, 3, 2, 2, 2, 2	2.1	2.6
3:40	89.8			1.8

In table 7 the change in reading is recorded on the line immediately below that on which is recorded the average activity producing such change. An analysis of the data presented in the case of this snail shows some interesting things. In the first case, the hour that showed an average activity of 2.5, as compared to the first hour, theoretically should have produced a change in reading of 2.6 instead of 2. The reason why it did not will be evident from a close inspection of the individual ten-minute records of activity. A change of 2.6 in reading did occur, however, during the fifth hour of the experiment in which the activity averaged 3.1. It was found (table 6) that activities varying between the degrees expressed by 2 and 3 would cause changes in carbon dioxide production that average about half of that found for activity 5. Evidently this same thing is true in the case of oxygen consumption.

Figure 1 shows how closely the change in the reading of the oxygen consumption in this experiment followed after the change in activity of the snail.

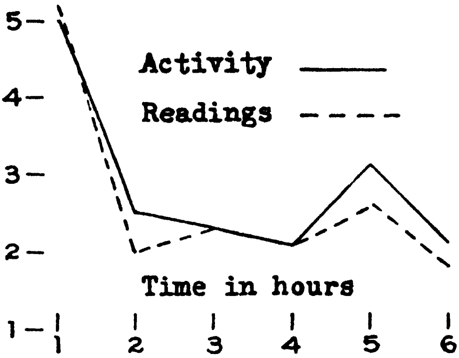

Fig. 1. Comparison of average activity and change in reading of table 7.

EFFECT OF PHYSIOLOGICAL STATE

That physiological state has a profound effect on the metabolism has been proved by the researches of Child (1919) on *Planaria dorotocephala.* He found that in starvation, the carbon dioxide elimination decreases rapidly the first few days and then falls off more slowly. However, after three or four months of starvation the carbon dioxide production is again equal to or greater than that at the beginning of the starvation period.

No experiments have been attempted by the writer on the effect of the physiological state of *Physa occidentalis* on its metabolism. It is believed that this factor has been adequately controlled so that it is unnecessary to take it into account in a mathematical sense in stating the effects of infection on the metabolic processes.

'Physiological state' is a term in bad repute. It is obviously somewhat difficult to define. It cannot be arrived at by inspection of the individual, nor can various modes of procedure be relied upon to reveal it—at least in *Physa.*

Complications arising from physiological states were avoided in so far as possible. Only material that appeared active, whether healthy or parasitized, and that had access to an abundance of food was used.

COMPARATIVE EXPERIMENTAL DATA

This section will deal with experimental results of a comparative nature. Although an attempt was made to control the factor of physiological state by the careful selection of material, this factor probably is not so easily controlled. The results as set forth in the following pages are undoubtedly modified by the physiological state; nevertheless, it is believed that they are sufficiently clear-cut to warrant the deductions that are made.

RELATIVE WEIGHTS

Faust (1917) observed that the mass of worms may be twice that of the digestive gland. Further, he said (1920, p. 81), ''A heavily infected snail may contain a far greater amount of parasites than its own net size or weight.''

In order to get at the relative weights of the mass of *Echinostoma revolutum* larvae in the tissues of *Physa occidentalis*, a few determinations of their actual weights in milligrams were undertaken. The snails were weighed whole with all superfluous water removed as noted previously. Following this, the shell was carefully removed, dried, and weighed. The weight of the soft parts was obtained by subtraction. By exceedingly careful dissection and teasing under the binocular dissecting microscope, the larval flukes were freed from the tissues of the host. During this operation a few cercariae were accidentally set free, but they were disregarded as they were too small to be handled in the subsequent procedure. However, no determinations were made in cases wherein more than a very few were set free. Nearly twenty snails were sacrificed for practice in removing the parasites intact in order to obtain those for which data are given. After the rediae were freed, they were all moved to the same place in the dish with a needle and picked up in a pipette. They were then allowed to settle in the pipette and were dropped with a minimum of water onto a sheet of filter paper. Following this they were quickly picked up with a specially prepared flattened needle under the binocular dissecting microscope and transferred to a small glass dish of water that had been previously weighed. This dish was made by cutting off a short piece of the end of a small vial. The gain in weight of this dish of water was recorded as the weight of the parasites. The net weight of the soft parts was obtained by a second subtraction. An unavoidable source of slight error is perhaps that of the amount of water that may be carried over by adhesion to the numerous rediae even though they are first dropped onto filter paper. The relative surface for adhesion is here far greater than in the case of the foot of the snail. At the most, this factor probably would not change the results more than a very small percentage, and would not modify the direction of the differences in weights which have been detected.

TABLE 8

RELATIVE WEIGHTS OF HOST AND PARASITE

No.	Total weight	Weight of shell only	Weight of soft parts including parasites	Weight of soft parts without parasites	Weight of parasites	Per cent parasites on total weight
36	0 1635	0 0406	0 1229	0 0973	0 0256	15 6
37	0 3738 (2 snails)	0 0582	0 3156	0 2354	0 0802	21 4
38	0 0621	0 0210	0 0411	0 0321	0 0090	14 4
39	0 1344	0 0382	0 0962	0 0838	0 0124	9 2

All five of these snails were individuals that could be considered 'heavily infected.' In fact, without exception they were seemingly infected to the limit of survival. The viscera in each case were completely riddled with rediae. Of the four cases given, the highest percentage of parasites was that in which they made up approximately one-fifth of the total weight. If the weight of the shell is disregarded, in this individual, the parasites bear the relation of 0.0802 : 0.2354, or nearly 1 : 3 to the net soft parts. The soft parts, of course, include the foot.

Some determinations were made of the relative weights of the whole animal, the shell only, and the soft parts only in healthy *Physa*. These results, taken in connection with those given above, point to some interesting things that seem to be more or less confirmatory of a point mentioned earlier in connection with the changes in the foot.

TABLE 9

RELATIVE WEIGHTS OF PARTS OF HEALTHY SNAILS

No.	Total weight in grams	Weight of shell only	Weight of soft parts (by subtraction)
40	0.4960 (4 snails)	0.0610	0.4350
41	0.2653	0.0410	0.2243
42	0.1722	0.0324	0.1398
43	0.2842 (2 snails)	0.0511	0.2331
44	0.1769	0.0360	0.1409
45	0 0824	0.0248	0.0576
46	0.2182	0.0373	0.1809
47	0.2357	0 0361	0.1996
48	0.1230	0.0282	0.0948
49	0.0886	0.0231	0.0655

The relations that the weights of the shells bear to the weights of the soft parts in the four cases listed in table 8 are approximately 1 to 4, 6+, 3, and 3+ respectively. Those of the ten cases listed in table 9 are 1 to 8+, 6+, 5+, 5+, 5—, 3+, 6—, 6+, 4+, and 3+ respectively. The average in the former case is 1:4; in the latter it is 1:5.1. This means that the soft parts, on the basis of the data in hand, are heavier in proportion to the weight of the shell in the healthy individuals. A possible explanation of this would be the reduction of muscular tissue in parasitism as suggested in a previous section.

Table 10 gives the dry weight of, and the percentage of water in the viscera in the cases of two analyses. Desiccation of the soft parts was accomplished by low heat for three hours with subsequent suspension over calcium chloride for ten days.

TABLE 10

DRY WEIGHT AND PERCENTAGE OF WATER IN THE VISCERA OF HEALTHY SNAILS

No.	Total weight in grams	Weight of shell only	Weight of soft parts wet (by subtraction)	Weight of soft parts (desiccated)	Weight of water (by subtraction)	Percentage of water
50	0.4960 (4 snails)	0.0610	0.4350	0.0827	0.3523	81.0
51	0.2653	0.0410	0.2243	0.0449	0.1794	80.0

Occasion will arise to refer to these figures later.

CARBON DIOXIDE ELIMINATION

For the determination of the carbon dioxide elimination in both health and parasitism in *Physa,* the hydrogen-ion method of Child (1919) (outlined on p. 332) was used. Each snail was placed in an individual tube with five cubic centimeters of water; the tube was then corked and sealed with paraffin. At the close of the experiment the tube was thoroughly shaken, uncorked, and the animal removed. The indicator was then added and the reading made immediately.

In all these experiments, the water used was taken from the same locality from which the snails had been collected. It was adjusted to a uniform hydrogen-ion concentration of 8.3 before use. This is 0.1 below the upper limit of the range of the indicator employed (phenolsulpholphthalein).

A control tube was set up together with each tube containing a snail and allowed to stand for the same length of time. In all these there was no change in the hydrogen-ion concentration during the short time of the experiments.

The time that each snail remained in its tube varied. This variation in time was due to the irregularities arising from the manipulations of removing the superfluous moisture from the snails and weighing them one after another as they were put into the tubes. Irregularities also arose when the readings were made. The time was recorded to the nearest minute. Six readings were made of the degree of activity of each individual.

No snails were used the average activity of which fell below the designation 2.5 for the reason stated in the preceding section. Snails that showed activity designation 1.0 at any reading were immediately discarded. Only snails that appeared fully active were chosen for these experiments.

A group that gave the appearance of being parasitized was picked out, as well as a group of healthy ones. For the first experiment ten of each were taken. In all cases the true condition of the snails was later proved by microscopic examination after the hydrogen-ion tests. A snail that appeared parasitized but that yielded no parasites, suggesting a condition of recovery from parasitism, was not used for data referable to the normal condition. In this first experiment all the ten snails chosen by chance as parasitized proved to be infected.

In subsequent experiments some had to be discarded. In these subsequent tests, small groups of snails that appeared parasitized were selected; those that were not proper material were rejected. The resulting cases were matched with a like number of healthy individuals. A total of fifty-six careful determinations were made on snails of known conditions. These, as implied immediately above, are divided evenly between the conditions of health and parasitism. Snails infected with cysts only, the cysts having come to light in individuals selected as healthy ones, were allowed to pass as healthy. The following tables present the data for these fifty-six *Physa*.

TABLE 11

CARBON DIOXIDE ELIMINATION OF PARASITIZED PHYSA OCCIDENTALIS

No.	Weight of snail	Time of test (minutes)	Average activity	pH		Change of pH	Coefficient
				Start	Finish		
52	0.1751	269	3.0	8.3	7.1−	1.2+	8.4
53	0.1682	270	3.0	8.3	7.0−	1.3+	9.5
54	0.1630	269	4.0	8.3	7.3−	1.0+	5.6
55	0.1570	221	4.0	8.3	7.2−	1.1+	7.8
56	0.1441	270	4.0	8.3	7.1−	1.2+	7.7
57	0.1139	267	4.0	8.3	7.2	1.1	8.9
58	0.0644	269	4.0	8.3	7.4	0.9	12.9
59	0.0920	260	4.0	8.3	7.3−	1.0+	10.3
60	0.0562	257	4.0	8.3	7.5	0.8	13.5
61	0.0712	255	3.7	8.3	7.3+	1.0−	14.8
62	0.0327	249	4.1	8.3	7.7	0.6	17.9
63	0.0264	240	5.0	8.3	7.7−	0.6+	18.6
64	0.0822	238	3.8	8.3	7.4+	0.9−	12.1
65	0.0961	232	4.0	8.3	7.3−	1.0+	11.1
66	0.1204	226	3.0	8.3	7.3−	1.0+	12.1
67	0.1122	212	4.6	8.3	7.1−	1.2+	10.9
68	0.0884	212	4.2	8.3	7.3	1.0	12.6
69	0.1219	266	5.0	8.3	7.2−	1.1+	6.6
70	0.1138	237	4.5	8.3	7.1	1.2	9.8
71	0.0242	233	5.0	8.3	7.5	0.8	27.7
72	0.0904	225	4.0	8.3	7.3+	1.0−	12.1
73	0.0708	217	3.5	8.3	7.3	1.0	18.5
74	0.0676	211	3.0	8.3	7.5+	0.8−	18.5
75	0.0514	208	3.5	8.3	7.5+	0.8−	21.3
76	0.0241	207	5.0	8.3	7.6+	0.7−	27.8
77	0.0308	225	4.0	8.3	8.0	0.3	10.7
78	0.0350	222	4.0	8.3	7.9	0.4	12.8
79	0.0344	219	4.0	8.3	7.9+	0.4−	13.2

TABLE 12

Carbon Dioxide Elimination of Healthy Physa occidentalis

No.	Weight of snail	Time of test (minutes)	Average activity	pH Start	pH Finish	Change of pH	Coefficient
80	0.1390	267	2.5	8.3	7.5−	0.8+	8.5
81	0.1000	268	3.0	8.3	7.5−	0.8+	9.8
82	0.0926	262	3.5	8.3	7.5	0.8	9.3
83	0.0906	233	3.0	8.3	7.7	0.6	9.3
84	0.0300	231	2.5	8.3	7.9+	0.4−	22.9
85	0.0737	220	2.5	8.3	7.7	0.6	14.6
86	0.0827	215	2.5	8.3	7.5+	0.8−	17.7
87	0.0853	199	3.0	8.3	7.5	0.8	15.6
88	0.0825	198	3.0	8.3	7.5	0.8	16.1
89	0.0337	196	4.0	8.3	7.8	0.5	18.9
90	0.1372	267	3.0	8.3	7.5−	0.8+	7.1
91	0.1461	270	3.2	8.3	7.5	0.8	6.3
92	0.1022	267	3.0	8.3	7.5	0.8	9.6
93	0.0720	266	3.0	8.3	7.7	0.6	10.2
94	0.1242	264	2.5	8.3	7.5−	0.8+	9.5
95	0.0967	259	3.1	8.3	7.7−	0.6+	7.6
96	0.0424	257	4.0	8.3	7.8−	0.5+	11.2
97	0.0677	254	2.5	8.3	7.8−	0.5+	11.5
98	0.0641	250	2.5	8.3	7.7+	0.6−	14.9
99	0.0372	245	3.0	8.3	7.9−	0.4+	14.3
100	0.0710	241	3.1	8.3	7.8	0.6	11.1
101	0.0743	236	2.8	8.3	7.8−	0.6+	12.0
102	0.0260	238	3.7	8.3	7.9	0.4	22.0
103	0.0292	229	3.5	8.3	7.9−	0.4+	16.8
104	0.0801	225	3.2	8.3	7.6	0.7	12.0
105	0.0680	245	3.0	8.3	7.8−	0.5+	10.0
106	0.0505	241	4.0	8.3	7.9	0.4	10.1
107	0.0775	238	3.0	8.3	7.9−	0.4+	7.2

The coefficients listed in the last columns of the above tables were obtained by use of the formula $\frac{C.T_R}{W.A}$ wherein C is the change in hydrogen-ion concentration, T_R the reciprocal of the time, W the weight, and A the average activity. Some hypothetical cases will make the derivation of this formula clear.

Case 1

Snail	Weight (mg.)	Time (minutes)	pH change	Average activity	Coefficient
1	100.0	240	1.0	5.0	1
2	100.0	240	1.0	2.5	2

In this case all the factors are equal except the average activity. Snail 2, having one-half the activity of snail 1, would be expected to produce carbon dioxide one-half as fast according to the data presented in connection with the effect of activity. However, since it produced the same pH change in this hypothetical case as the more active snail, it is evident that its coefficient should be double that of snail 1. It will be clear, then, that in the formula the value of the average activity will stand below the line, indicating that it is to be used as a divisor. Applying the above formula, the coefficients become 1 and 2 respectively for the two snails.

CASE 2

Snail	Weight (mg.)	Time (minutes)	pH change	Average activity	Coefficient
3	100.0	240	1.0	5.0	1
4	100.0	240	2.0	5.0	2

In this case, all the factors are equal except the rate of change of the hydrogen-ion concentration. The snail that produces the greater change should have the greater coefficient. From this it follows that the numerical expression of the change should stand in the relation of a dividend instead of a divisor. As a consequence it goes above the line in the formula.

CASE 3

Snail	Weight (mg.)	Time (minutes)	pH change	Average activity	Coefficient
5	100.0	240	1.0	5.0	1
6	50.0	240	1.0	5.0	2

Here weight is the variable factor. A snail of half the weight of another but producing the same amount of carbon dioxide, other factors being equal, should have a coefficient twice as great. Hence weight assumes the relationship of a divisor in the formula.

CASE 4

Snail	Weight (mg.)	Time (minutes)	pH change	Average activity	Coefficient
7	100.0	240	1.0	5.0	1
8	100.0	120	1.0	5.0	2

Within limits, the time is directly proportional to the change produced. From this it follows that the time also may be used as a divisor. The same result, however, is obtained if the reciprocal of the time is transferred to the other side of the equation, and the formula becomes less cumbersome.

The data presented in tables 11 and 12 in summary, give the following figures:

TABLE 13

SUMMARY OF DATA IN TABLES 11 AND 12

Cases	Average time (min.)	Average activity	Average change of pH	Average weight	Average coefficient
Healthy (28 cases).......... ...	242	3.06	0.61	0.0777	12.36
Parasitized (28 cases)........	238	3.99	0.9	0.0867	13.34

By chance the parasitized snails of this group averaged heavier than the healthy ones. These tests were performed in the fall of the year. At this time the surviving parasitized snails seem to be, on the average, heavier for the reason that the lighter ones are perhaps more vulnerable and die off more quickly. This same thing has been noted when parasitized snails were kept in aquaria. These figures, however, refer only to actual weights. The relative weights, as shown before, are greater in the cases of healthy individuals. This greater average weight of the parasitized snails would have the tendency to reduce the average coefficient of this group. The greater activity would have the same effect.

The average coefficient, however, is greater for the parasitized group. This would seem to indicate that the relative carbon dioxide production is greater in the parasitized condition.

Some attempts were made to test the carbon dioxide production of the parasites themselves. The parasites that were dissected from the snails listed in table 8 were used for this purpose. The results of these experiments were unsatisfactory since the rediae began to disintegrate before the termination of the tests. The contained cercariae, however, survived in good condition. In the main, it appeared that the parasites produced relatively more carbon dioxide per unit weight than the host. They were physiologically younger animals and such a result might therefore be expected.

The factors concerned in a study of carbon dioxide production such as the above are so many and so variable that it is extremely difficult to draw critical conclusions. That more carbon dioxide, proportionately, is produced by parasitized snails seems certain. The proportion of this produced by the host and by the parasites themselves cannot be accurately determined on the basis of the data in hand because of the early death of rediae on removal from the host. It is a possibility that the metabolism of the host is increased per unit weight; on the other hand, it may be decreased according to the weight and age of the infection, increasing in early and decreasing in later stages of the infection. The amount of respiring tissue is less in a parasitized snail of the same gross weight as a healthy one. This, as pointed out before, is due to the relatively greater weight of the shell in parasitized individuals and also to the fact that the parasites themselves add considerably to the gross weight. A decrease in the metabolic rate of the host, then, may be either actual or apparent. It may be due to a real decline in the respiratory rate; or it may be due to the fact that there is less snail tissue present to respire, as noted above. In either case, the metabolic products of the parasites, added to those of the host in proportionately greater amounts, would be responsible for the general rise in carbon dioxide elimination. An increased rate of metabolism in this residuum of host tissue added to the higher rate of carbon dioxide production by the larval flukes would bring about a marked advance in the total carbon dioxide production of the parasitized snail.

OXYGEN CONSUMPTION

Snails were selected for tests of their oxygen consumption in much the same manner as for the hydrogen-ion tests described above. Only one snail could be handled at a time in the Thunberg-Winterstein apparatus. A larger apparatus, designed on the same plan and intended for testing large numbers of snails at a time, was constructed. This apparatus was not used because it was recognized that tests on *individual snails* would yield more accurate results for comparative purposes than those on larger numbers of snails. One could not be assured at the outset that *all* members of a group being tested at one time were either healthy or parasitized. In any one given group selected for either type, some might be and some might not be parasitized. This would complicate the problem. When one snail is used, its condition may subsequently be ascertained and the results are applicable to the class to which it belongs.

The readings on the millimeter scale of the apparatus were multiplied by the calibration value and corrections made for barometric pressure, temperature, and vapor tension. All readings were thus reduced to standard conditions; namely, 0° C., 760 mm. of mercury. The volumes thus obtained were expressed as fractions of a cubic centimeter. These values were each multiplied by the factor 1.42 to reduce them to milligrams.

The method used for measuring the oxygen consumption is not so erratic as the hydrogen-ion method employed for measuring the carbon dioxide production. It is by far a more satisfactory type of procedure.

A total of fifty determinations were made on the oxygen consumption of *Physa* at room temperature. These are evenly divided between healthy and infected individuals. The following table gives the data for the healthy ones

TABLE 14

OXYGEN CONSUMPTION OF HEALTHY SNAILS

No	Weight in grams	Time in hours	Average activity	Milligrams of oxygen
108	0.1141	4	3.0	0.0502
109	0.1546	4	3.6	0.0675
110	0.1211	4	4.0	0.0630
111	0.1150	4	3.2	0.0348
112	0.1540	4	3.2	0.0648
113	0.0823	4	3.0	0.0239
114	0.0779	4	3.8	0.0316
115	0.0861	4	3.5	0.0376
116	0.0874	4	2.6	0.0256
117	0.0648	4	3.6	0.0356
118	0.0765	4	3.8	0.0360
119	0.1011	4	3.1	0.0298
120	0.0710	4	2.6	0.0247
121	0.0691	4	3.6	0.0221
122	0.0824	4	4.0	0.0296
123	0.0768	4	3.0	0.0266
124	0.1621	4	4.0	0.0654
125	0.0721	4	3.2	0.0310
126	0.1482	4	3.2	0.0591
127	0.0776	4	4.5	0.0342
128	0.0690	4	4.0	0.0210
129	0.0627	4	4.0	0.0223
130	0.0962	4	3.5	0.0289
131	0.0682	4	4.0	0.0220
132	0.0800	4	4.0	0.0276

Table 15 presents the data obtained in the cases of the parasitized individuals.

TABLE 15

OXYGEN CONSUMPTION OF PARASITIZED SNAILS

No.	Weight in grams	Time in hours	Average activity	Milligrams of oxygen
133	0.1702	4	4.0	0.0672
134	0.0771	4	3.0	0.0301
135	0.0820	4	2.6	0.0277
136	0.0872	4	3.4	0.0365
137	0.1024	4	3.5	0.0344
138	0.0787	4	3.0	0.0269
139	0.0724	4	3.8	0.0349
140	0.0821	4	2.5	0.0280
141	0.0733	4	3.4	0.0246
142	0.1462	4	3.6	0.0710
143	0.1322	4	3.8	0.0694
144	0 1242	4	3.2	0.0411
145	0.0712	4	3.6	0.0381
146	0.0728	4	3.8	0.0322
147	0.0840	4	4.5	0.0372
148	0.0830	4	4.5	0.0283
149	0.1026	4	3.5	0.0279
150	0.0682	4	4.0	0.0246
151	0.1247	4	4.2	0.0647
152	0.1206	4	3.2	0.0388
153	0.0900	4	4.0	0.0299
154	0.0727	4	3.2	0.0327
155	0.0620	4	3.6	0.0350
156	0.0811	4	4.0	0.0300
157	0.1462	4	4.0	0.0686

The averages for the two above tables are as follows:

TABLE 16

AVERAGES FOR TABLES 14 AND 15

Snails	Weight in grams	Time in hours	Average activity	Milligrams of oxygen
Healthy (25 cases)........	0.0948	4	3.5+	0.0366
Parasitized (25 cases)........	0.0962	4	3.5++	0.0392

The parasitized snails each averaged 1.4 mg. heavier than the healthy ones. As stated before, this fact is meaningless from the standpoint of actual relative weights of the two classes of snails. The absolute weights found are probably functions of season and mortality. Contrary to the results found in the case of carbon dioxide elimination, the average activities of the two classes of individuals are here approximately equal. There is still, however, a slight balance in favor of the parasitized group. The relative oxygen consumption, according to the above data, is approximately 7 per cent greater for the parasitized snails than for the healthy group. It seems then that, as in the case of carbon dioxide elimination, oxygen consumption is also increased by the diseased condition. It is yet to be determined, however, what parts the host and parasites take respectively in bringing about this result.

The greatest body of data that has been presented with regard to the respiratory exchanges of a pulmonate is that of Hesse (1910). This author made a large number of determinations under varying conditions, but not from the standpoint of health and parasitism by trematode larvae. He worked with *Helix pomatia,* a comparatively large terrestrial form.

In one table, Hesse records data for the oxygen consumption of the form he worked with varying from 13.49 to 62.59 milligrams per kilogram-hour with the average close to 45 milligrams. The highest figure he records is 77.52 milligrams per kilogram-hour. Six cases selected at random from those given for *Physa occidentalis* (tables 14 and 15, above) give the following results:

TABLE 17

MILLIGRAMS OF O_2 CONSUMED BY PHYSA PER KILOGRAM-HOUR

Snail referred to	Milligrams O_2 per kilogram-hour
109	109.1
118	117 6
129	88.9
133	98.7
142	121.4
148	85.2
Average.. .	103 4

The oxygen consumption of *Physa occidentalis* appears to be approximately but little more than twice as great as that of *Helix pomatia* per unit weight and unit time. A condition of this kind may be expected since *Physa* is a much smaller and more active animal than *Helix.*

RESPIRATORY QUOTIENTS

The respiratory quotients of *Physa occidentalis* were determined by a modified technique in which the Thunberg-Winterstein apparatus was used in connection with an adaptation of Lund's (1919) method of titration for the measurement of the carbon dioxide.

Only one animal was used in each of these experiments. It was placed in one of the chambers of the apparatus in a small amount (½ to 1 cc.) of water from the aquarium. Water from the same source was run into the other chamber until the level in both was the same. Both chambers were then closed with ground glass stoppers, each having on its lower end a small cup to carry the alkali used to absorb the carbon dioxide. Before the stoppers were put into place, a small amount of a saturated solution of barium hydroxide was placed in each cup. This amount was the same in each case and was very accurately measured. The entire operation was carried through for one stopper and then repeated for the other one. By so doing, the time the barium hydroxide was exposed to the air was the same in each case. These operations were performed in a current of fresh air from an open window and the amount of exposure of the alkali was timed in each case in order that uniformity would be insured. That this exposure would have no appreciable effect on the results is evident. The exact degree of concentration of the alkali is not important; it is important that both cups of the apparatus in any one experiment contain alkali of the *same* concentration.

After the above manipulations had been completed, the apparatus was put into the water bath and the stop cocks opened. In from one to two minutes equilibrium was reached and the stop cocks closed. The experiment was then allowed to run for four hours. During this time the activity of the snail was watched as was described before.

At the expiration of the four-hour period the amount of oxygen consumed was read off by noting the position of the oil droplet in the tube connecting the two chambers as compared with its position at the outset. The apparatus as a whole was removed from the water bath and wiped. Each stopper with its cup was quickly removed and transferred to a specially prepared vial for titration of the alkali. The time of exposure was here also made the same in each case in order that error arising from this source may be avoided.

These vials used for titration were large ones; they were about six inches in length and an inch in diameter. Each was closed with a rubber stopper through which a hole had been bored. Each hole was plugged with a short section of glass rod. Inside each vial, a smaller vial was suspended by a thread in such a way that it could be quickly removed when the stopper was taken out. These smaller vials were half filled with the concentrated alkali solution in order that the larger vials might be rendered free of carbon dioxide.

The vials were prepared two hours before needed. They were thoroughly cleaned and a uniform amount of indicator (brom-thymol blue) was put into each. The small vials of alkali were lowered into them and the stoppers pushed in tight. A few minutes before use, the stoppers were momentarily removed and the small vials taken out. The glass stoppers of the Thunberg-Winterstein apparatus were next transferred one to each vial with the precautions noted above. The water in each chamber of the respirometer was transferred to the proper vial by means of a pipette. Only one operation of the pipette was necessary for each transfer. It was put into the vial through the opening in the stopper, the glass rod being removed for this purpose.

The contents of each vial were next titrated. The acid was introduced through the hole in the stopper by the agency of a capillary tube fastened to the tip of the burette with a short section of gum rubber tubing. Titration was carried forward in each vial to near the end point. Then the vials were alternately manipulated, with readings on the burette appropriately recorded, until the contents of both were identical in color and remained identical after shaking and standing. Save when acid was being introduced each vial was kept tightly closed with a glass rod.

The difference in the amount of titrable alkali in the two vials represented the amount of carbon dioxide given off by the snail. The normality of the acid (HCl) was carefully checked before each experiment against a standard solution of sodium hydroxide freshly prepared for each test. The acid was kept in stock at approximately N/100 strength. It was diluted to N/500, checked, and used.

This method is reasonably accurate. Several tests were made of the amount of carbon dioxide recoverable from the decomposition of known amounts of sodium carbonate. Four hours were allowed for absorption of the carbon dioxide by the alkali. The error averaged

—4.6 per cent. In the case of measurement of the carbon dioxide produced by the snail, this substance is evolved so slowly that it is probably absorbed by the alkali as fast as it appears.

The water in both the chamber containing the snail and in the control chamber of the apparatus was transferred to the vials along with the alkali cups for titration for the reason that part of the carbon dioxide remains in solution. This was evidenced in a number of tests by the fact that the water in the chamber with the snail at the end of the four-hour period had a much lower pH reading than at the start. It is obvious that a state of equilibrium between the air and the water in the chamber with respect to carbon dioxide would not be attained under the conditions of the continued presence of the snail.

Twenty-nine respiratory quotients were obtained for various conditions of *Physa occidentalis.* These are recorded below.

TABLE 18

RESPIRATORY QUOTIENTS OF HEALTHY PHYSA OCCIDENTALIS—FALL

No.	Weight in grams	Milligrams of oxygen	Milligrams of carbon dioxide	Time in hours	Average activity	Respiratory quotient	Temperature of environment
158	0.1034	0 0641	0 0462	4	5.0	0.72	21° C.
159	0.0693	0 0438	0 0323	4	4.5	0 73	21° C.
160	0.0663	0.0357	0 0231	4	3.6	0 64	22° C.
161	0.1086	0.0406	0 0341	4	3.2	0.84	19° C.
162	0 1714	0.0463	0.0403	4	2.5	0.87	17° C.
163	0.1299	0.0398	0.0375	4	2.6	0.94	21° C.
164	0.0152	0.0215	0 0238	4	2 6	1.10	21° C.
165	0.1386	0.0376	0.0304	4	3.0	0.80	20° C.

TABLE 19

RESPIRATORY QUOTIENTS OF HEALTHY PHYSA OCCIDENTALIS—WINTER

No.	Weight in grams	Milligrams of oxygen	Milligrams of carbon dioxide	Time in hours	Average activity	Respiratory quotient	Temperature of environment
166	0.1300	0.0213	0.0094	4	2 8	0.44	4° C.
167	0.0846	0.0172	0.0087	4	2.6	0.50	7° C.
168	0.1162	0.0221	0.0106	4	3.0	0.48	4° C.
169	0.0732	0 0188	0.0069	4	2.6	0.36	4° C.

TABLE 20

RESPIRATORY QUOTIENTS OF HEALTHY PHYSA OCCIDENTALIS—SPRING

No.	Weight in grams	Milligrams of oxygen	Milligrams of carbon dioxide	Time in hours	Average activity	Respiratory quotient	Temperature of environment
170	0.0851	0.0253	0.0192	4	3.0	0.76	19° C.
171	0.1544	0.0681	0.0577	4	4.0	0.85	21° C.
172	0.0800	0.0321	0.0205	4	3.8	0.64	21° C.
173	0.0742	0.0309	0.0278	4	3.2	0.90	22° C.
174	0.0816	0.0360	0.0262	4	4.5	0.73	20° C.

TABLE 21

RESPIRATORY QUOTIENTS OF PARASITIZED PHYSA OCCIDENTALIS—FALL

No.	Weight in grams	Milligrams of oxygen	Milligrams of carbon dioxide	Time in hours	Average activity	Respiratory quotient	Temperature of environment
175	0.1291	0.0688	0.0619	4	3.8	0 90	18° C.
176	0.0907	0.0272	0.0212	4	2.5	0.78	17° C.
177	0.1444	0.0717	0.0659	4	3.8	0.92	20° C.
178	0.0624	0.0321	0.0309	4	4.2	0.96	22° C.
179	0.0621	0.0214	0.0158	4	3.2	0.74	22° C.
180	0.0790	0.0302	0.0260	4	4.0	0 86	20° C.
181	0.1665	0.0649	0.0521	4	4.0	0.80	18° C.
182	0.0708	0.0351	0.0246	4	3.8	0.70	20° C.
183	0.0799	0.0273	0.0245	4	2.5	0 90	18° C.
184	0.1002	0.0280	0.0248	4	3.5	0.88	19° C.
185	0.0808	0.0271	0.0252	4	2.8	0.93	19° C.
186	0.0816	0.0262	0.0249	4	2.5	0.95	19° C.

In tables 18–21 the last column records the temperature of the water from which the snails were taken at the time they were collected. Those snails taken in the fall and spring were collected from shallow water in protected spots in the lakes in Golden Gate Park and kept in aquaria in the laboratory at room temperature for short periods before use in the tests. Those taken in the winter were tested immediately with the apparatus surrounded with water kept at the temperature of the environment from which the snails came by means of ice.

These tables give averages as follows: healthy snails, fall, 0.83; healthy snails, winter, 0.44; healthy snails, spring, 0.77; parasitized snails, fall, 0.86.

According to Starling (1920) the respiratory quotient of man is not greatly affected by moderate muscular activity not associated with dyspnoea. The amounts of carbon dioxide and oxygen involved, however, are much increased with activity but their relative proportions are not much altered. Since the degrees of activity exhibited by the snails in the above tests lie in the usual range, it is perhaps the case that the respiratory quotients of the snails also are little affected by this condition. The vital factor concerned in a modification of the respiratory quotient is the diet. According to Starling (1920) fats and proteins in the diet lower the quotient. A carbohydrate diet would tend to produce a quotient of unity due to the fact that the oxidation of such food materials produces carbon dioxide in the same volume as the oxygen used up. This same author said that toward the end of summer the marmot eats large quantities of carbohydrate food and lays on large quantities of fat. Under such conditions the respiratory quotient may go as high as 1.5. This is due to the liberation of oxygen in the synthesis of fat. Less oxygen, as a result, need be taken in. During hibernation the quotient sinks quite low, often as low as 0.2.

The low quotients obtained for *Physa occidentalis* in the winter were obtained near the end of a prolonged cold period during which the temperature of the water often approached the freezing point. The snails were to be found in a quiescent condition under stones and leaves. Since the winter climate of the San Francisco Bay district is relatively mild, perhaps the condition of *Physa occidentalis* during this period is one approaching pseudo-hibernation. Even in the tests at the same low temperature they roused themselves considerably.

That there is a tendency in *Physa* to build up fat deposits in the fall of the year is indicated by the fact that in at least one individual (no. 164) the quotient went above unity. The general trend of the quotients, however, would indicate a mixed diet.

The quotients of the parasitized snails average but three points above those of the healthy ones for the same time of year. This is scarcely a difference of sufficient magnitude to be considered significant. The direction of difference, however, is of significance. Such a difference would be expected from the nature of the data on carbon dioxide production and on oxygen consumption given in former sections. The relative increase of the former in parasitism is greater than that of the latter according to the data that have been presented.

A number of authors have given respiratory quotients for various gastropod mollusks. Thunberg (1905*a*) recorded quotients of 0.9–1.0 for *Limax agrestis*. Meyer (1925) found a quotient of 0.6 for *Bulimus detritus*, and 0.22 for *Cyclostoma elegans*. Vernon (1897) in two tests on *Helix pomatia*, among many tests on other cold-blooded animals both vertebrate and invertebrate, found quotients of 0.74 and 0.79. He said that carbon dioxide production increased regularly with the temperature (in the case of *Helix*) up to 20° C. Then it suddenly diminished for a few degrees, with a slight increase from 22.5 to 30° C. That less carbon dioxide is produced by *Physa occidentalis* at lower temperatures may be seen by reference to the values for the winter snails (table 19) as compared to the values in the other tables. Hesse (1910) recorded a variety of respiratory quotients for *Helix pomatia* under variable conditions. The following are typical:

TABLE 22

DATA OF HESSE ON HELIX POMATIA

Condition of animal	Quotients	Temperature
Active	0.59–0.74	13.4°–15.6° C.
Inactive	0.60–1.00	13.0°–16.6° C.
Active	1.17–2.23	2.5°– 3.2° C.
Inactive	1.14–1.97	2.95°– 4.05° C.

These data of Hesse for *Helix pomatia* are hard to reconcile with the data obtained by the writer for *Physa occidentalis*. It must be borne in mind, however, that the two snails differ widely, the former being a comparatively large terrestrial form, the latter a small aquatic form, each with a rather different type of feeding and food.

OTHER METABOLIC PRODUCTS

A limited number of tests were made of the fat content and the carbohydrate content of the tissues of both healthy and parasitized *Physa occidentalis*. The results of these experiments lend support to the conclusion that may be arrived at by an examination of sections of these two types of snails that are stained to show the relations of the two above-mentioned substances.

Quantitative determinations of the fat content were made by the ether-extraction method as described in the section dealing with materials and methods. Since *Physa occidentalis* is a relatively small snail, each experiment was performed with from five to twenty-five individuals. Each snail was examined to determine whether or not it was infected before it was used. They were weighed in groups, shell and all, with all superfluous moisture removed.

Table 23 gives the percentages of fat found in ten experiments.

TABLE 23

PERCENTAGES OF EXTRACTABLE FAT IN PHYSA OCCIDENTALIS

Experiment	Gross weight of snails in grams	Weight of extracted fat in grams	Per cent of fat	Condition of snails
1	0.7824 (5 snails)	0.0217	02.77	Parasitized
2	1.3900 (10 snails)	0.0300	02.15	Healthy
3	0.9970 (6 snails)	0.0208	02.08	Parasitized
4	0.6734 (5 snails)	0.0225	03 30	Parasitized
5	1.2560 (10 snails)	0.0273	02.17	Healthy
6	1.0752 (10 snails)	0.0270	02 51	Healthy
7	1.1966 (10 snails)	0.0335	02.80	Parasitized
8	0.5859 (5 snails)	0 0154	02.63	Parasitized
9	0.7056 (5 snails)	0.0171	02.42	Parasitized
10	0.8248 (5 snails)	0.0180	02.18	Healthy

The average percentage of extractable fat in the healthy group is 02.25; *that in the parasitized group is* 02.66. *This gives a balance* of 00.41 per cent preponderance of the latter over the former, or an increase of 18.2 per cent. In the above *tests, the rediae* were not dissected out of the parasitized groups of individuals. The fat content as determined in these cases includes that of both the snails and their parasites. Part of this increased fat content in parasitized snails may be due to a relatively greater amount of fat in the parasites themselves. That part of it represents an actual proportionate increase of this substance in the tissues of the host, especially in the hepatopancreas, is evidenced by the very marked fatty degeneration that occurs in the tissues of the snail in many cases in parasitism. Microscopic evidence for the increase of fat in parasitized snails was discussed earlier in this paper and figured in figures 2 and 9, plate 16.

The groups upon which experiments for the determination of the carbohydrate content were performed were given a preliminary examination and weighed in the same way as those for the fat determinations. The snails of each group were thoroughly ground in repeated changes of small amounts of boiling distilled water. Clean quartz was used in the mortar. The resulting mixture was filtered, and repeated small portions of boiling distilled water were poured over the residue in the filter. This process probably does not remove absolutely all the glycogen from the tissues but since all the experiments were performed with proportionate amounts of boiling water, the comparative results are not modified to any appreciable extent by this factor.

The final extract was boiled with about 5 per cent of concentrated HCl added. This was then allowed to cool and was neutralized exactly with sodium carbonate solution. That most, if not all, the resulting sugar was glucose was determined by using the left-over solution of one of the quantitative tests. This was allowed to concentrate by moderately quick evaporation and characteristic crystals of glucozazone were obtained by the use of phenylhydrazine.

The quantitative determinations were made by the technique of Benedict (1911). An amount of the quantitative solution was used that would be exactly decolorized by one milligram of glucose. This was placed in a very small porcelain crucible on an electric hot plate. The extract was dropped into the boiling reagent very slowly from a suspended burette at such a rate that the volume of liquid in the crucible was kept nearly constant. The amount of carbohydrate present in the extract as a whole was easily determined by noting the proportion of the whole to the part that was required to decolorize the standard quantity of Benedict's reagent.

Table 24 sets forth the data obtained in the experiments on the carbohydrate content of seventy-one snails in five groups.

TABLE 24

PERCENTAGES OF CARBOHYDRATE IN PHYSA OCCIDENTALIS

Experiment	Gross weight of snails in grams	Milligrams of carbohydrate	Per cent of carbohydrate	Condition of snails
1	2.3870 (15 snails)	12 36	00 51	Healthy
2	0 8170 (6 snails)	3.0	00 36	Parasitized
3	2.6406 (25 snails)	4 48	00 17	Parasitized (rediae removed)
4	1 4320 (15 snails)	3.44	00 24	Parasitized (rediae removed)
5	1.4767 (10 snails)	9 47	00 64	Healthy

The above figures support the microscopic findings in that the amount of carbohydrate (largely glycogen) is reduced in the parasitized condition. In the second experiment the rediae were not removed from the snails. The percentage of carbohydrate in this case is lower than in the cases of the healthy animals but not so low as in the third and fourth experiments. Removal of the rediae was accomplished prior to the last two experiments. These facts would seem to indicate that the carbohydrate content of the net host tissue is much reduced in the parasitized state. They also indicate that the carbohydrate content of the parasites themselves is considerable.

Jordan (1913) recorded the fact that *Helix pomatia* contains 5.650 grams of glycogen per kilogram of weight. This would give a percentage of 00.56 which well agrees with the figures in the above table.

The same author described an experiment in which *Limax variegatus* was starved 21 days and then fed with bread for 24 hours. At the end of this time 1.55 grams of the digestive glands contained .052 gram or 3.38 per cent of glycogen; 18.2468 grams of the rest of the body tissues of the animals used in this experiment contained 0.0641 gram or 0.35 per cent of glycogen.

Undoubtedly a relatively greater percentage of glycogen occurs in the hepato-pancreas of *Physa occidentalis* than in the rest of the body. Since this organ is the major seat of infections with larval trematodes, it is perhaps here that the most profound reduction in the amount of glycogen actually takes place.

GENERAL CONSIDERATIONS

A variety of experiments in addition to those reported in the foregoing pages have been carried out. In the main these have led to no results of a conclusive nature. They are reported here because in some instances at least they are suggestive of interesting possibilities.

The black pigment granules referred to before and apparently described as an "antidote" by Agersborg (1924) are probably the same as the chromatophores of Leydig (1850 and 1876). The pigment figured by Semper (1857) likewise falls in the same category.

This pigment in *Physa occidentalis* is similar in appearance to that figured by the above authors in the mollusks with which they worked. It is evidently a melanin-like substance and closely allied to like pigments found in the invertebrate and vertebrate groups in general. The description given by Dawson (1920) for the coloring matter in the integument of *Necturus maculosus* agrees in many points with the nature and behavior of that of *Physa occidentalis.*

In the latter animal the pigment granules are most abundant in the integument of the mantle and the dorsal portion of the foot. They are not lacking, however, in the inner tissues of the body. The subcutaneous tissues, especially in the cases of mature healthy animals, are often heavily loaded with this substance. It appears in the connective tissue of the hepato-pancreas and in the connective tissue of the lamellae of the kidney. Often the basement membrane of the kidney lamellae is the seat of a dense agglomeration of these particles. This latter fact suggests that they are in some measure excretory products. That they are such is also supported by the manner of their excretion and replacement in the epidermis. Turchini (1922) suggested a similar function for the melanin particles in the ink sac of the squid.

Dawson (1920), in a review of the literature on the subject, mentioned several possibilities with regard to the origin of such pigment. It may be of mesenchymal origin or it may be deposited in the cells of the epidermis by wandering cells. On the other hand, the epidermal cells may be capable of forming their own pigment. Another possibility is that the pigment cells are modified leucocytes. In *Physa occidentalis* the granules are both intracellularly and extracellularly disposed. They may even occur within the muscle fibers (pl. 17, fig. 16), a fact noted by Agersborg (1924). Polygonal cells with clear nuclei and dense zones of pigment granules occur in the integument. They are often to be seen within the Leydig cells in the connective tissue of the foot and other regions of the body.

Turchini and Ladreyt (1921) suggested a mitochondrial origin of such granules. Agersborg (1924) thought them to be intranuclear in origin. No observations have been made by the writer that would throw light on this subject.

Like melanin, the black pigment granules of *Physa occidentalis* are not soluble in water, alcohol, ether, chloroform, or acids. They are promptly bleached by the chlorine of the potassium chlorate-hydrocloric acid-50 per cent alcohol mixture. Following such bleaching, acid dyes such as eosine seem to have no effect upon the granules even when applied for extended periods. Polychrome stains such as Wright's and Giemsa's stain do not recolor them. Prolonged action of a basic dye such as Heidenhain's haematoxylin apparently will give a suggestion of staining in some cases. The presence of the granules in smears made from the integument may be revealed (with proper illumination) as clear and colorless particles even after bleaching. The unbleached granules stand out deeply black. This would indicate a compound nature for these bodies.

A few experiments were undertaken to determine the effect of parasitism upon the amyloclastic and proteoclastic action, to borrow the terms of Bayliss (1919), of the enzymes of *Physa occidentalis*. The method of Wohlgemuth (1908) was used for the former; that of Hawk (1916) for the latter. No results were obtained that could be regarded as significant. It is felt, however, that this is a good example of the unreliability of test-tube methods as an index to what is going on in an animal. It was pointed out before that the general eosinophilic reaction of the tissues of the snail was greatly enhanced by parasitism, especially the epithelium of the hepato-pancreas. That this condition is without effect on the efficiency of the enzyme complex does not seem reasonable. The effect of such a condition would not be revealed in test-tube experiments wherein the reaction is adjusted to slight alkalinity.

During the course of these experiments one interesting possibility came to light. Extracts of parasitized snails for obtaining the enzymes were ordinarily made after the removal of the rediae. In some cases wherein extracts were made of snails with their contained parasites *in situ,* such extracts showed a stronger autolytic activity for the snail protein in solution than in cases where the parasites were removed. This would seem to indicate that the parasites do possess ferments capable of a solvent action on the tissues of the host.

The subject of phagocytosis has been thoroughly studied in the gastropods and in the invertebrates as a group. In fact in the invertebrates, this seems to be the main line of defense against foreign bodies. Experiments in which living *Bacillus tuberculosis,* avian type, were injected into the viscera of *Physa occidentalis* in amounts ranging from 0.0001 to 0.001 cubic centimeter were performed by the writer. By use of the same apparatus, but with a somewhat larger micro-needle, living cercariae of *Echinostoma revolutum* were injected into *Physa occidentalis* and into *Lymnaea* sp. Smears in the former case showed that the characteristic phagocytic reaction took place. In the latter case, apparently little or no phagocytic action took place when the cercariae were injected into *Physa occidentalis.* This is the normal host of this larval fluke. Under ordinary conditions, the cercariae would encyst—perhaps without calling forth any marked response. However, in the case of those injected into *Lymnaea* sp., in two out of five cases a very marked phagocytosis was evident.

It is possible that specificity as a phenomenon of parasitism, as well as immunity, is partly a function of phagocytosis. That a larval fluke, the miracidium for instance, will fail to develop unless it enters the proper host may be due in part to the possibility that in other hosts phagocytic action is more pronounced.

Some attempts were made to test the relative degrees of total nitrogen eliminated by both healthy and parasitized snails according to the methods outlined on page 334. It was felt that the conditions of these experiments were not adequate and that reliable results were not obtained because of the fact that no measure of the rate of evacuation of the kidney could be determined. It is known that the mollusk empties its nephridial cavity at more or less lengthy intervals according to its physiological state (Cuénot, 1894, *et al.*). Since such variations were undoubtedly a factor in the case of *Physa occidentalis,* the results obtained could have but little value for comparative use.

However, in the experiments performed there was an indication that more nitrogen was eliminated by parasitized snails than by healthy ones but a quantitative statement would be unwise for the reasons stated. That more nitrogen is eliminated by the diseased individuals is to be expected in view of the nature of the microscopic findings in the kidneys of such animals.

GENERAL DISCUSSION

There can be do doubt that many changes are produced in *Physa occidentalis* by the presence of the larvae of *Echinostoma revolutum.* These changes are manifested in a twofold way. The tissues and their component cells of this snail are modified in diverse ways from the structural point of view. Their functions are also changed to a considerable degree. The net result is one of definite structural and functional damage.

As mentioned before, Ward (1907) and later Brumpt (1922) have pointed out that the effects of parasites on the host may be mechanical, morphological, and physiological. The morphological changes in *Physa occidentalis* may be exemplified in such reactions as the abnormal proliferation of the connective tissue of the hepatopancreas and the formation of capsules of host tissue about the invading parasites. Attendant upon these is a whole series of more or less far-reaching changes in the hepato-pancreatic epithelium, the gonad, the kidney, the cells of the alimentary epithelium, the connective tissue in general, and the musculature. The sum total of these changes tends to make the life-processes of the parasitized animal more difficult of normal accomplishment.

The physiological modifications are expressed in the limitations placed on the normal physiological processes of the host. The respiratory exchanges undergo changes in keeping with the accelerated metabolic rate that goes with the parasitized condition; absorption, storage, secretion, and excretion are profoundly affected.

In order to function in an efficient way, the tissues and organs of an organism must carry on the appropriate metabolic activities in a manner that may be characterized as normal. It will be evident that in the case of an individual snail whose body is invaded by other organisms this is no longer possible. The metabolism and metabolic products are distinctly augmented.

The parasitized *Physa occidentalis* must provide food not only for itself but also for the larval flukes that infest its vital organs.

As a result it has to work harder for a living than a normal snail. That this is literally the case is borne out by the fact that infected snails are often seen to feed more voraciously than normal ones. Those that appear to be in a state of stupor and rouse themselves only occasionally to feed invariably succumb to the infection when their last resources of resistance are overcome.

The parasites not only feed at the expense of the host but they also delegate to it the duty of disposing of their own excretory wastes. The host must dispose of these as well as its own wastes. Undoubtedly the excretory products of the parasite have a more harmful effect on the host than the mere requisitioning of food materials. The latter would only tend to increase the burden of digestion and absorption and, at the most, result in a malnourished condition of the snail. The former, however, may well prove actually poisonous and in time result in a general weakening of the infected individual as the sequel to the burden thrown upon its excretory mechanisms, the hepato-pancreas and the kidney.

Throughout the process of parasitism of *Physa occidentalis* by the larvae of *Echinostoma revolutum* many reactions of both local and general natures have been noted. All these point to the influence of a soluble toxin, or toxins. That the excretory products of the parasites play such a rôle cannot be doubted. These produce a distinctly abnormal reaction in the hepato-pancreatic epithelium. The accumulation of excretory material of both host and parasite in the excretory vacuoles of the hepatic cells ultimately leads to a condition wherein such products show lytic properties for the cells. Disintegration or necrosis results. That harmful products of a secretory nature are also liberated by the parasites is evidenced by the fact that the mature cercariae produce a substance capable of digesting the host's cells, particularly the cells of the connective tissue of the sinuses of the hepato-pancreas. These cells are digested *in situ* and are evidently not taken into the alimentary tracts of the cercariae. The nuclei of these cells resist digestion to the last and may be found free in a structureless mass of débris. Part of this débris may, as a matter of fact, enter the digestive tract of the parasite; but the disintegrative digestive process takes place prior to this and outside the body of the parasite. Such digestive secretions, together with the waste products thrust upon the host by the parasite, are perhaps responsible in greatest degree for the pathological changes, both morphological and physiological, that have been described in the preceding sections.

The degree to which *Physa occidentalis* can tolerate infections with *Echinostoma revolutum* is truly remarkable. This may, in the main, be said of trematode infections in mollusks generally. The immunity factors involved in such cases have never been demonstrated. The ability of the snail to withstand a condition of parasitism in which the major portion of its viscera is replaced by parasites is probably an expression of the innate vitality of the individual and the action of specific substances called forth to counteract the harmful action of the products of the parasites. The nature of these substances that may be elaborated for the defense of the snail remains in doubt. Their existence, however, is hardly a matter for dispute in view of the very nature of the case. After all, the processes of immunity are probably universal (Cantacuzene, 1923) in application—differing only in minor details of circumstances of elaboration and operation. The question of ultimate immunity; that is, the question whether or not snails that have lost their infection may be reinfected, remains open. The balance of evidence, however, points to such an immunity. Snails have been found that gave every evidence of having been parasitized but contained no active parasites at the time of examination. Such snails were found both with and without the encysted cercariae. Some of these cysts are cercariae that never left the snail in which they developed; some have immigrated from other snails. This latter process is, however, the more common (Johnson, 1920). Since such snails, harboring encysted cercariae only, were in an environment that presented ample opportunities for reinfection with miracidia, but were not so infected, an immunity factor that bars the ciliated larvae but offers no resistance to the cercariae must be present (Sewell, 1922).

The most profound changes produced in *Physa occidentalis* are those set forth in the foregoing pages as occurring in the hepatopancreas. This organ is the seat of infection with the multiplicative phases of the parasite and as such might well be expected to show marked changes. The pathological picture induced in the gastropod hepato-pancrease by fluke infection is on the whole very suggestive of the condition set up in the vertebrate liver infected with pathogenic organisms. Hypertrophy of the interstitial tissue is common to both. Stasis may be produced in the ducts of both by pressure. Another result common to both is ultimate necrosis of the pathological cells. Neumann (1892) described the process of fatty degeneration of the cells of the liver of the sheep infected with the common liver fluke. That fatty degeneration also occurs in the snail infected with larval flukes seems certain from the nature of the data set forth in the previous pages. Another common effect of the liver fluke upon the sheep and other animals is an anemic condition (Rivas, 1920; Brumpt, 1922). A condition perhaps comparable to this is seen in the snail in the marked reduction in pigment and in the weight of the heavily infected individuals.

That the encysted cercariae imbedded in the tissues of the gastropod are not pathogenic is the concensus of opinion of the authors. In the case of *Physa occidentalis,* the writer has found many individuals heavily loaded with encysted cercariae without any evidence of pathological conditions. In a great many cases the agamodistomes were accumulated in greatest number in non-vital organs such as the collar. They may occur, however, in great numbers in various regions of the body, including the hepato-pancreas, the gonad, the kidney, and even under the epithelium of the alimentary tract in the region of the buccal cavity. A common location is the musculature of the foot. In all cases, sections of snails harboring cysts only, present an essentially normal picture of the various organs. Relatively heavy infections with the encysted cercariae in the hepato-pancreatic connective tissue sinuses do, as a matter of fact, produce considerable crowding of the cells of the tubules and noticeable stasis in the lumen. That this crowding by the cysts does not produce profound changes would seem to indicate that such pathological changes as do occur in the presence of the earlier and non-encysted parthenogenetic larvae are due to the metabolic and toxic relationships of the parasites to the host and in no large degree to mechanical crowding.

The cycle of infection proposed on page 340 differs from that of Agersborg only in that it does not include the "antidote" of this author. The writer believes that Agersborg has given an undue significance to the normal pigment granules of the snails with which he worked. That an "antidote" or antitoxin, to use the recognized term, is elaborated by the host in some measure can scarcely be doubted. However, it is not a substance visible as pigment and can only be revealed by suitable biological procedures.

It is of advantage to both the host and the parasite that a condition of balance be established. By such a condition, both are able to propagate and maintain themselves. When the cumulative effects of the relationship overstep the bounds of resistance, the snail dies and in so doing defeats the parasites. In general the association of the gastropod and its trematode parasites may be said to be well balanced but undoubtedly of much individual and racial detriment to the host. The effects on the host, both immediate and progressive, are in the main of such nature that the host is able to live and function after a fashion, succumbing only in extreme cases or under abnormal environmental conditions of an unfavorable nature.

SUMMARY AND CONCLUSIONS

1. Non-parasitized individuals of *Physa occidentalis* are usually much darker in color than parasitized ones. Parasitized ones are dirty grayish in color and often a peculiar unnatural orange color in the region of the viscera. This color reaction is a result of at least two causes: (*a*) the reduction of the pigment of the host in the parasitized condition, and (*b*) the pigmentation of the parasites themselves. Snails appearing abnormal often show no parasites, suggesting a condition of recovery from a previously parasitized state.

2. We have found 83.6 per cent of 550 *Physa occidentalis* from Golden Gate Park in San Francisco infected with the larvae of *Echinostoma revolutum*. The degree of infection varied profoundly in individual cases from a few parasites to a condition wherein the viscera were completely riddled with the larvae. Snails infected with encysted cercariae only, and in varying degrees of such infection, were found. The encysted stage is apparently of little or no pathological significance.

3. The parasites in their multiplicative phases are always found in the sinuses of the connective tissue, particularly in the hepato-pancreas in the case of active larvae. The encysted flukes may be found in any tissue. The fact that the active larvae are found in the sinuses of the hepato-pancreas suggests that they arrive at this location by way of the circulatory channels after their initial penetration into the host.

4. The cycle of infection is fundamentally an annual one with minor variations due to favorable conditions of temperature. Cercariae are more abundant in the fall of the year.

5. The muscular tissue of the foot is often reduced in parasitized individuals and the glycogen content of this organ is lowered. The pigment granules are decreased in quantity.

6. The "antidote" of Agersborg is to be identified with the normal pigment material.

7. The hepato-pancreas consists of racemose tubules, the epithelium of which is comprised of two general types of cells. This organ is the major seat of trematode infection in the snail.

8. The main structural changes to be noted in the hepato-pancreas in parasitism are: (*a*) The fat content as revealed by reagents is increased in some cases; (*b*) the glycogen content is decreased; (*c*) necrosis of the pathological cells follows augmentation of the excretory products; (*d*) excretory products are greatly increased in the cells of the hepatic epithelium and exercise a lytic effect. The final necrotic mass is loaded with eosinophilic material; (*e*) the nuclei of the cells of the hepato-pancreas resist lysis; they are found to migrate from the typically basal position in the cell to a position more nearly median between the base of the cell and the lumen of the tube; (*f*) pressure produced by the masses of parasites occasionally all but closes the lumen of the gland; and (*g*) the lime cells are more resistant to disintegration than the other type of cell of the digestive gland.

9. The amoebocytes are largely suppressed in cases of heavy infection.

10. The kidney is more heavily loaded with concretions in parasitism. Necrotic areas often occur in this organ. The nuclei of the renal cells often present more granular and less distinct karyosomes than in healthy animals. This last is probably the result of the toxic secretions or excretions of the parasites that are located elsewhere than in the kidney.

11. The Golgi bodies are increased in size and number in the epithelium of the buccal cavity in parasitism. The reverse occurs in the intestine. Very little change is apparent in this material in the stomach and gizzard.

12. Hypertrophy and fibromata occur in the connective tissue of the hepatic organ. In later stages some atrophy occurs here as well as in the foot. Motile cercariae often digest *in situ* the cells of the host; the nuclei of these cells resist this digestion and may be found free in a structureless mass of débris.

13. The gonad is reduced or completely destroyed.

14. The heaviest case of infection found was one wherein the parasites made up approximately 20 per cent of the total weight.

15. Older snails metabolize more rapidly than younger ones in an absolute sense but not in a relative sense. The metabolism is directly proportional to the degree of activity of the individual.

16. Relatively more carbon dioxide is eliminated by parasitized snails. The coefficients found averaged 12.36 and 13.34 for the healthy and parasitized groups respectively. This is an increase of 7.9 per cent of the latter over the former.

17. Relatively more oxygen is consumed by infected individuals. On the average, the healthy animals each consumed 0.0366 milligram of oxygen in a four-hour period. In a like time the parasitized ones consumed 0.0392 milligram of oxygen, or an increase of 7.1 per cent over that consumed by the healthy snails.

18. The respiratory quotients show a tendency to be increased in parasitism. This increase in the quotients in the parasitized animals averages but .03, or 2.4 per cent higher than the quotients of the healthy group. The quotients in normal animals appear to be higher in the fall of the year than in the winter and spring, averaging 0.83, 0.44, and 0.77 respectively.

19. Quantitative determinations show an increase in the fat content in certain cases and a decrease in the reducing sugar content in the condition of parasitism. In the material studied, the average percentage of fat in healthy animals was 2.25 per cent; that in the parasitized ones was 2.66 per cent, an increase of 18.2 per cent. The highest percentage of sugar found in healthy material was 0.64 per cent; the lowest found in parasitism was 0.17 per cent.

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EXPLANATION OF PLATES

All figures are camera lucida drawings or photomicrographs from sections of healthy or parasitized *Physa occidentalis.* The parasites are the larvae of *Echinostoma revolutum.* The technique and the magnification are stated in the descriptions of the individual figures.

PLATE 16

All figures are camera lucida drawings.

Fig. 1. A lamella from the kidney of a heavily infected snail. Large concretions in the vacuoles of the renal cells. Granular pigment in the sinus. Compare with figure 3. Silver-hydroquinone process. × 720 diameters.

Fig. 2. A group of cells from the hepato-pancreas of a healthy snail. Note fat globules and two types of vacuoles. Nuclei indistinct. Compare with figure 9. Mann-Kopsch method. × 720 diameters.

Fig. 3. A lamella from the kidney of a healthy snail. Small concretions in the vacuoles. Granular pigment in the sinus. Three amoebocytes in the sinus. Silver-hydroquinone process. × 720 diameters.

Fig. 4. A group of three cells from the renal epithelium of an infected snail. Indistinct and granular karyosomes. Heidenhain's haematoxylin and eosin. × 1375 diameters.

Fig. 5. A group of cells from the renal epithelium of a healthy snail. Distinct karyosomes and distinct basement membrane. Heidenhain's haematoxylin and eosin. × 1375 diameters.

Fig. 6. Ciliated epithelium of posterodorsal wall of the buccal cavity of a healthy snail. Golgi bodies few and distal to nuclei. Mitochondria indistinct. Compare with figure 7. Da Fano's modification of Cajal's method. × 720 diameters.

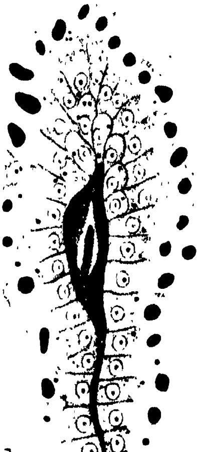
Fig. 7. Ciliated epithelium of posterodorsal wall of the buccal cavity of a parasitized snail. Golgi bodies numerous and distal to nuclei. Mitochondria indistinct. Da Fano's modification of Cajal's method. × 720 diameters.

Fig. 8. Portion of a hepato-pancreatic tubule. Three groups of lime cells with large spherical nuclei and with distal ends of cells crowded with lime granules. The hepatic cells show small oval nuclei and two types of vacuoles. The excretory granules show as separate particles in the more distal vacuoles; the secretory vacuoles lie basal to median and contain a single mass of material. Distinct basement membrane and pseudo-cuticula. From healthy material. Heidenhain's haematoxylin and eosin. × 345 diameters.

Fig. 9. A group of cells from the hepato-pancreatic epithelium of a parasitized snail. Note fat globules and two types of vacuoles. Fat more abundant than figured in figure 2. Part of parasite figured underlying basement membrane and in sinus. Mann-Kopsch method. × 720 diameters.

Fig. 10. Six nuclei lying free in the sinus of the hepato-pancreas. Masses of structureless débris of cytoplasm of cells. Heidenhain's haematoxylin and eosin. × 720 diameters.

Fig. 11. Two lime cells from an infected snail. Note large agglomerations distal to nuclei. These agglomerations are disintegration products of the lime granules. Heidenhain's haematoxylin and eosin. × 720 diameters.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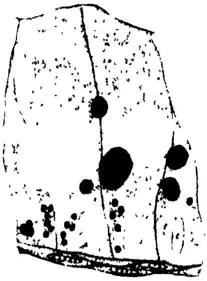
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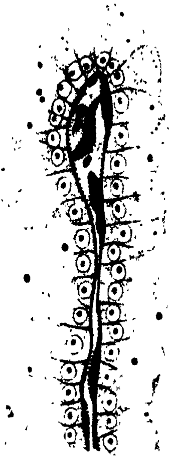
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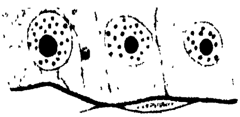
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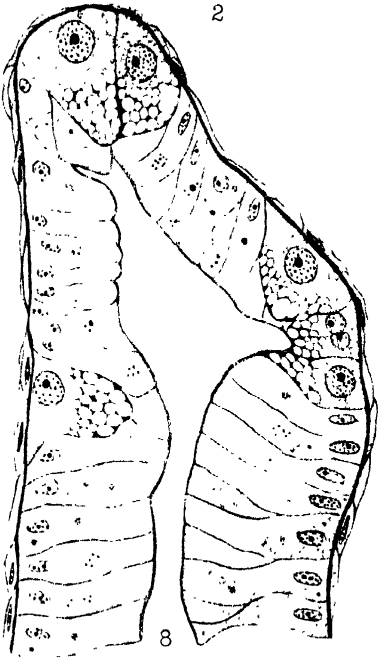
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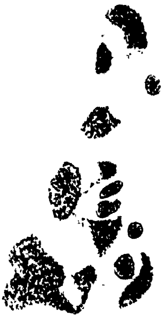
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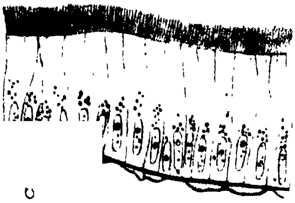
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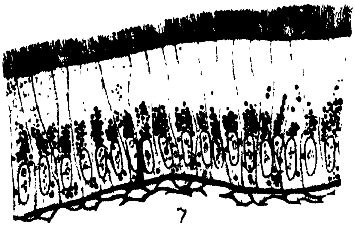
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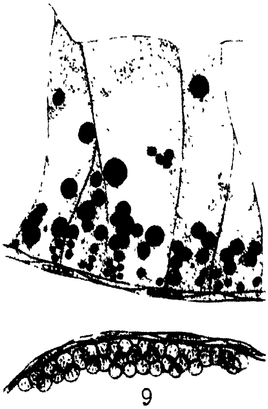
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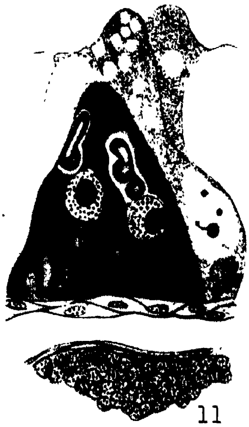
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PLATE 17

All figures are photomicrographs.

Fig. 12. Section of viscera of a healthy snail. Gonad and liver tissue intact. Heidenhain's haematoxylin and eosin. × 40 diameters.

Fig. 13. Section of the viscera of a parasitized snail. Gonad completely replaced by parasites. Necrotic liver tissue may be seen in the upper part of the figure. Heidenhain's haematoxylin and eosin. × 40 diameters.

Fig. 14. Muscular tissue of the foot of a healthy snail. Pigment granules abundant. Heidenhain's haematoxylin and eosin. × 400 diameters.

Fig. 15. Muscular tissue of the foot of a healthy snail. Pigment granules abundant. Heidenhain's haematoxylin and eosin. × 200 diameters.

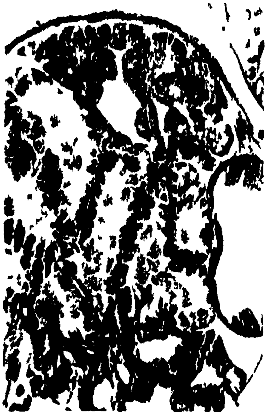
Fig. 16. Muscular tissue of the foot of an infected snail. Pigment granules relatively absent. Note a few granules within the muscle fibers. Da Fano's modification of Cajal's method. × 400 diameters.

Fig. 17. Muscle tissue of foot of an infected snail. Note atrophy of tissue. Heidenhain's haematoxylin and eosin. × 72 diameters.

Fig. 18. Muscle tissue of foot of a healthy snail. Note well developed nature of the tissue. Heidenhain's haematoxylin and eosin. × 72 diameters.

Fig. 19. A group of lime cells from the hepato-pancreas of an infected snail. Lime cells largely intact. Nuclei have migrated away from basal position. Parasite underlying basement membrane and in sinus. Heidenhain's haematoxylin and eosin. × 400 diameters.

Fig. 20. General nature of the hepato-pancreas of an infected snail. Augmented excretory vacuoles. Heidenhain's haematoxylin and eosin. × 72 diameters.



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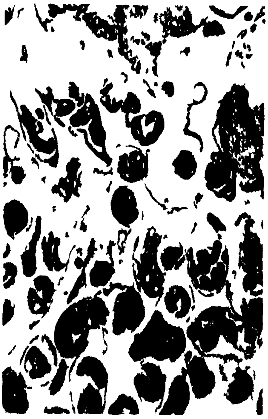

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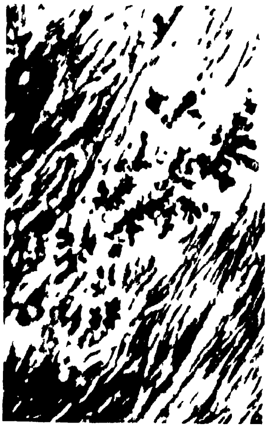

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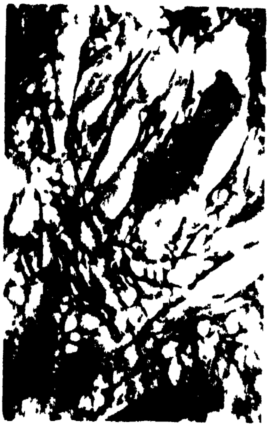

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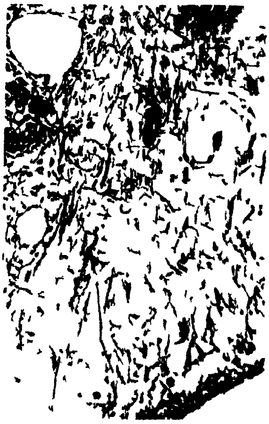

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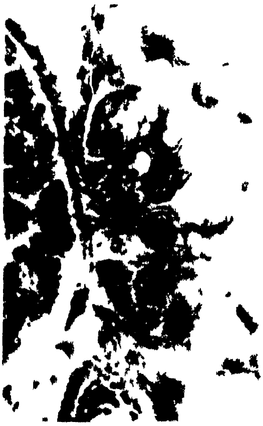

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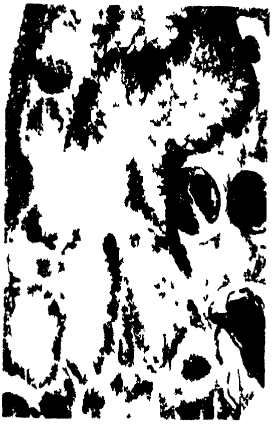

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PLATE 18

All figures are photomicrographs.

Fig. 21. Epithelium of mid-intestine. Cilia partially denuded. Golgi bodies few. Nuclei indistinct. Parasite underlying the epithelium. Da Fano's modification of Cajal's method. × 400 diameters.

Fig. 22. Portion of epithelium of the stomach of an infected individual underlaid with parasites. Heidenhain's haematoxylin and eosin. × 400 diameters.

Fig. 23. A portion of figure 22 more highly magnified. Note dispersed nature of the chromatin of the nuclei. Heidenhain's haematoxylin and eosin. × 620 diameters.

Fig. 24. Portion of epithelium of stomach of a healthy snail. Note distinct karyosomes of nuclei. Heidenhain's haematoxylin and eosin. × 620 diameters.

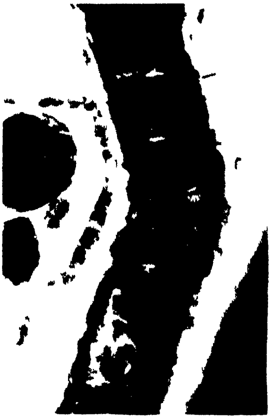
Fig. 25. Portion of epithelium of gizzard of an infected snail. Parasites in connective tissue. Paranuclear bodies granular and well developed. Da Fano's modification of Cajal's method. × 400 diameters.

Fig. 26. Portion of epithelium of gizzard of a healthy snail. Paranuclear bodies granular and well developed. Da Fano's modification of Cajal's method. × 400 diameters.

Fig. 27. Hepato-pancreatic epithelium of a parasitized snail. Paranuclear bodies basal. Nuclei do not show. Da Fano's modification of Cajal's method. × 400 diameters.

Fig. 28. Necrotic hepato-pancreatic epithelium underlaid with parasites. Note intact condition of nuclei. Heidenhain's haematoxylin and eosin. × 400 diameters.

Fig. 29. Three lime cells from infected snail. Note distinct nuclei and distinct lime granules. A few of the latter lie free of the cells due to being displaced in sectioning. Compare with figure 19. Roehl's haematoxylin method. × 750 diameters.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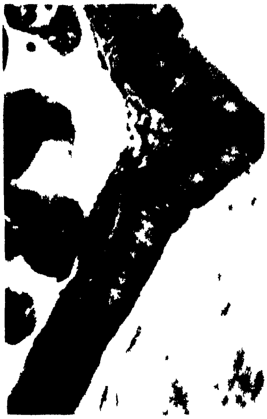
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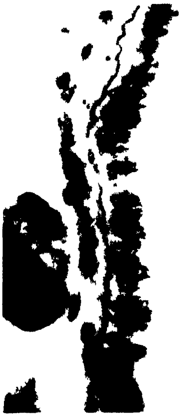
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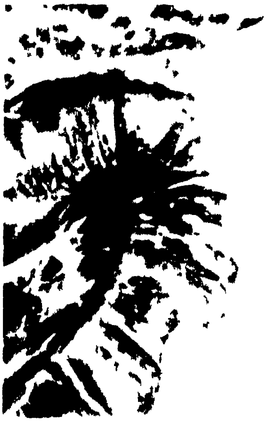
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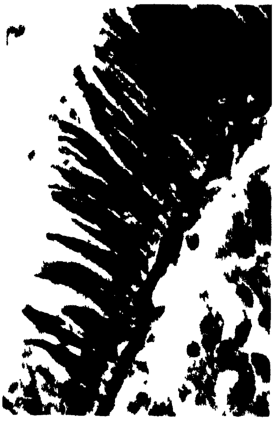
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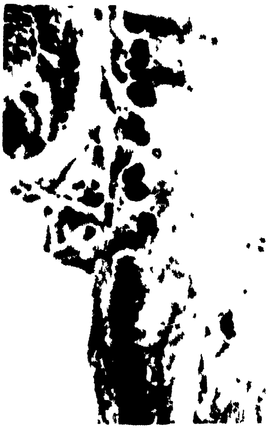
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PLATE 19

All figures are photomicrographs.

Fig. 30. General nature of the kidney of an infected snail. Note necrotic condition and numerous large concretions. Silver-hydroquinone method. × 400 diameters.

Fig. 31. General nature of the kidney of a healthy snail. Note well organized condition and small concretions. Silver-hydroquinone method. × 400 diameters.

Fig. 32. Single lamella from the kidney of a healthy snail. Note foam-like nature of the excretory vacuoles. Concretions small. Numerous amoebocytes in sinus. Heidenhain's haematoxylin and eosin. × 400 diameters.

Fig. 33. Muscle tissue from foot of a healthy snail. Note amoebocytes. Photographed from a thin portion of the section where amoebocytes are more easily differentiated from cross-sections of muscle fibers. Heidenhain's haematoxylin and eosin. × 400 diameters.

Fig. 34. Muscle tissue from foot of a parasitized snail. Amoebocytes absent. Heidenhain's haematoxylin and eosin. × 400 diameters.

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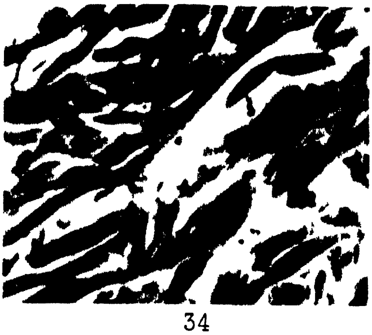

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THE NEUROMOTOR APPARATUS OF BOVERIA TEREDINIDI NELSON, A CILIATE FROM THE GILLS OF TEREDO NAVALIS

BY

EDITH A. PICKARD

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INTRODUCTION

The observations in this paper grew out of an attempt to discover if there were any parasites present in the body of *Teredo navalis* from San Francisco Bay. An examination of the digestive organs of this mollusk has never shown the presence of any ciliate. Within the gill chamber, however, three ciliates have been found.

The main object of this investigation has been to work out the morphology and describe the neuromotor system of one of these, *Boveria teredinidi* Nelson. In a later paper I hope to give an account of the life histories of the other two ciliates.

ACKNOWLEDGMENTS

The writer wishes to make due acknowledgment of her indebtedness to Dr. Charles A. Kofoid under whose direction this study has been made. Some of the teredos used in this investigation were procured through the courtesy of Mr. F. D. Mattos of the Southern Pacific Railway Company. I am also indebted to Mr. S. Yabroff for his advice in the use of the Da Fano silver-gold method as a stain for ciliate Infusoria.

REVIEW OF LITERATURE

The genus *Boveria* was established by Stevens (1901) for a heterotrichous marine ciliate found in the respiratory organs of *Holothuria californica.* One species, *Boveria subcylindrica,* was described in her paper.

Later the same author (1903) described a variety, *Boveria subcylindrica* var. *concharum,* from the bivalves, *Tellina exigua* and *Capsa fragilla* from the Bay of Naples. A form from Japan has been described as a new species, *Boveria labialis,* from the respiratory tree of *Stichopus japonicus* and the gill-lamellae of a *Tellina* species by Ikeda and Ozaki (1918). A preliminary report was published by Nelson (1923) on a new species, *Boveria teredinidi,* from the gills of *Teredo navalis* and *Bankia gouldi* of Barnegat Bay, New Jersey. The above citations indicate the work that has been done up to the present time on this most interesting genus.

OCCURRENCE AND GEOGRAPHIC DISTRIBUTION

The *Boveria* used in this study came from teredos infesting wood in various parts of San Francisco Bay. Most of the preserved teredo material in the collection of the Department of Zoology of the University of California showed an infestation of *Boveria* of greater or less degree. Fresh material for study of these living ciliates and for the preparation of slides was obtained in part from old piling at Crocket, through the courtesy of Mr. A. A. Brown, and from wood from the Southern Pacific Company's laboratory. Since only a few specimens of *Bankia gouldi* were obtained, and these were but lightly infested, all our descriptions, figures, and drawings are of *Boveria* from *Teredo navalis.*

My observations confirm those of Nelson (1923) that *Boveria* are confined entirely to the gills of the host. The number to be found in a single host may be few or many. In general, it may be said that they appear in teredos of medium and large size. In the fall of 1923 and spring of 1924 the material obtained from the laboratory at the pier of the Southern Pacific Railway Company showed a heavy infestation, while hosts obtained from piling in open water in the fall of 1925 and spring of 1926 harbored very few of the ciliates. However, the larger numbers may represent simply a sporadic case rather than an indication of a rhythmical seasonal cycle.

It would appear that the genus *Boveria,* like many other ciliates, is of more or less cosmopolitan distribution, having been reported from both the Atlantic (Nelson, 1925) and Pacific coasts (Stevens, 1901), the Bay of Naples (Stevens, 1903, 1910), and from the waters of Japan (Ikeda and Ozaki, 1918).

We are unable to contribute any explanation as to the method of transfer from one host to another. Our observations have not revealed the presence of the protozoan in the water of the aquarium in which wood infested by teredos was kept. While this might seem to indicate that they do not escape through the siphons, it is by no means conclusive. They may be liberated only at certain times or they may make their escape in such small numbers that they have failed of detection in centrifuged water. On the other hand, it may be possible that *Boveria* might infect the young embryos of the host previous to their discharge.

RELATIONSHIP TO THE HOST

Our present study has given no evidence that *Boveria teredinidi* produces any lethal or even pathogenic effects on its host. This observation agrees with that expressed by Stevens (1903) and Nelson (1923) in their studies.

Boveria teredinidi has been observed only in the gill chamber of its host and its nutrition appears to be entirely holozoic. The food consists, in part, of diatoms taken from the incurrent water, but the bulk of the contents of the food vacuoles consists of epithelial and leucocyte cells from the body of the host. These cells appear to be present as waste products and not as a result of injury to the gill cells by *Boveria.* There is no secreting adhesive area at the aboral end of the ciliate. The aboral end of the organism is only in casual contact with the epithelial cells of the host and when disturbed the ciliate swims rapidly away. The position of contact is maintained by a vigorous stroke of the cilia in the direction of the peristome.

Ikeda and Ozaki (1918) have found that *Boveria labialis* invades the subepithelial connective tissue of the respiratory tree of the host and encysts therein.

A study of three sets of sections of the gill chambers of *Teredo* shows no evidence of such tissue invasion and parasitism by *Boveria teredinidi* in the case of *Teredo navalis.* It may be possible, however, under certain conditions not yet observed that *Boveria teredinidi* may also be found to behave in a similar manner.

SYSTEMATIC POSITION OF GENUS AND SPECIES

The family to which *Boveria* belongs was not fully determined by Stevens (1903), who placed it in the order Heterotricha. Until this year, so far as we have been able to determine, no investigator has attempted to establish its exact position among Ciliata.

Calkins (1926) classifies *Boveria* under the Holotrichida, crediting it to the family Microthoracidae, and also includes it in the family Pleuronemidae.

When we consider the sum total of the distinguishing characteristics of *Boveria,* the evidence would seem to show a closer relationship to the Heterotricha. The very pronounced oral and the conspicuous spiral of adoral cilia are not distinctive characters of the Holotrichida but of the Heterotricha.

The fact that the mouth and pharynx are rarely open except during the ingestion of food might seem to stand in the way of the inclusion of *Boveria* in the Heterotricha. We may, however, consider this structure not as primarily, but rather as secondarily closed, and therefore place *Boveria* with the Heterotricha.

The prominent spiral *pharyngeal fiber* found in the pharynx suggests that possibly here is a ciliate which has lost its undulating membrane in the mouth and that this structure is represented only by this persistent fiber of the neuromotor system in the pharynx.

The morphology and the mode of division of *Boveria* show many characteristics suggestive of the Stentoridae. However, while myonemes are present, the spiral in *Boveria* is dexiotropic in the sense of a right-handed screw which in order to penetrate must be turned from left over to right. There are other distinctive differences, to be described later, which make it impossible to fit this ciliate into any existing family.

We propose that *Boveria* be removed from the order Holotrichida where it was placed by Calkins (1926) to the order Heterotrichida and that a new family be established to be known as Boveridae, and to have the following characteristics.

BOVERIDAE Fam. Nov.

Body form truncate or conical; adoral cilia forming a dexiotropic spiral; no somatic ciliation within the peristomal field; one central macronucleus and one micronucleus located near the aboral end during the interphase between fissions; mouth and pharynx not permanently open; no ciliary membrane in the pharynx, but a persistent neuromotor pharyngeal fiber. One contractile vacuole near the peristome. Marine forms: ectoparasitic; usually attached to the gills or respiratory organs of the host by cilia of aboral end. Reproduction by binary fission and also by conjugation.

Inasmuch as the genus *Boveria* has not figured extensively in literature, we submit here a summary of the two described species and one variety.

Comparison of *Boveria subcylindrica* sp. nov., *Boveria subcylindrica* var. *concharum* Based on Stevens (1901, 1903, 1910), *Boveria labialis* Based on Ikeda and Ozaki (1918), and *Boveria teredinidi* Nelson, Based on Our Findings

Boveria subcylindrica	*Boveria subcylindrica* var. *concharum*	*Boveria teredinidi*	*Boveria labialis*
Light attachment to membrane of respiratory tree of host	Same to gills	Very light attachment to epithelial cells of gill lamellae of the host	Light attachment to membrane of respiratory tree of host or to gill lamellae
Easily detached, swims rapidly in slightly spiral course, aboral end foremost, with slight flexion of oral end of body	Same	Same	
Body elongate, varying from cylindrical form to that of truncated cone	Same	Truncate; subconical	Body trumpet-shaped
Aboral end rounded	Aboral end pointed	Aboral end rounded	Aboral end rounded
Length of body 54 to 81 microns	27 to 102 microns on *Tellina exigua*, 65 to 121 microns on *Capsa fragillis*	Length of body 26.9 to 172.8 microns. Breadth at oral end 11.5 to 30.7 microns	Length of body 31 to 100 microns. Breadth at oral end 16 to 26 microns
Peristomal spiral double row of long coarse cilia united at ends	Same	Adoral ciliary spiral double parallel dexiotropic ending dorsally in a leiotropic spiral	Peristomal spiral double row of long coarse cilia
Spiral forms turn of 360° and 290° of second turn	Spiral forms turn of 360° and 200° of second turn	Spiral forms one and a quarter turns	Spiral forms turn of 360° and 290° of second turn
Pellicle thin, flexible, yet difficult to rupture	Same	Same	
Mouth at 'posterior' end within loop formed by union of 2 rows of oral cilia at inner end of spiral	Same	Mouth at anterior end surrounded by oral cilia extended from oral ring	Mouth depression in center of peristomal field

Boveria subcylindrica	*Boveria subcylindrica* var. *concharum*	*Boveria teredinidi*	*Boveria labialis*
Somatic ciliation arranged in parallel longitudinal rows. Slightly oblique	Same	Same	Same
20–27 rows of cilia	Same	18–22 rows of cilia	20–26 rows of cilia
Oral cilia one-half length of body	Same	Oral cilia one-third to one-fifth length of body	Oral cilia one-third length of body
One contractile vacuole posterior to peristome	Nearer to outer end of spiral	Near outer end of spiral on dorsal side	One contractile vacuole
Macronucleus oval; centrally placed in interphase between fissions	Same	Same	Same
Micronucleus near aboral end in interphase between fissions	Same	Same	Same
Lenticular disc between micronucleus and aboral end	Not present	Not present	Not present
Division by oblique fission Atrophy of mouth and peristome at onset of division	Same	Division by binary fission. Dedifferentiation of mouth at onset of division	Division by binary fission
New peristome arises as lateral band	Same	Arises from portion of migrated adoral fibers	
Micronucleus has four distinct chromosomes	Same	Not determined	Four distinct chromosomes
No lip at outer end of peristomal disc	Same	Same	Conspicuous lip 10–15 microns long

STUDY OF LIVING ORGANISMS

The host, *Teredo navalis,* was carefully removed from the burrow in the wood in which it was found and placed in sea water. When the gill cavity is opened and examined under a binocular dissecting microscope, *Boveria,* if present, will be found with its posterior end in contact with the gills and the body extending radially out into the lumen of the tube. This position is maintained by the vigorous stroke of the somatic cilia directed toward the peristome. When disturbed, it swims rapidly away.

The *Boveria* may then be placed on a slide by making a light smear, adding a little of the fluid of the gill chamber or sea water, and sealing the coverglass with vaseline to prevent too rapid evaporation of water. When thus observed they often appear gregarious, collecting about food particles. I have not been successful in my attempts to get *Boveria* to take in carmine particles. This may indicate a selective choice of food. Food vacuoles within the endoplasm are found usually in the anterior half of the body; only occasionally are they to be found posterior to the macronucleus.

The body shows remarkable flexibility although a characteristic form is maintained at all times. *Boveria* always swims with the aboral end foremost with a slight flexion of the oral part of the body. The course is slightly spiral, which, viewed from the aboral end of the animal, may be described as counter-clockwise or from right over to left.

Boveria teredinidi was described by Nelson (1923), being distinguished by him from *Boveria subcylindrica* on the following specific differences: "(1) the presence of short and of elongated forms, designated respectively as types truncata, length 33 to 35 microns; diameter at oral end 18 to 22 microns; (2) very light attachment to the epithelium of the host; (3) adoral cilia more than twice the length of the body cilia; (4) very abundant food vacuoles, 1 to 40." This author makes no mention of other specific characters associated with the variation in the length of the axes, which ordinarily are used in creating types.

In order to determine how nearly our animals agree with those of Nelson (1923), we have carefully measured a series of individuals. It is not practical to attempt to measure a large number of living

Boveria because of their ceaseless activity, hence preparations were used when making our measurements. No animals in stages of division were included, following the plan of Stevens (1901). Cilia were not included. The body length and breadth at the oral end of four hundred Boveria were measured. Following the method used with *Balantidium* by McDonald (1922) we have computed the ratio of the length to the breadth. These ratios were graphed with the results shown in figure A.

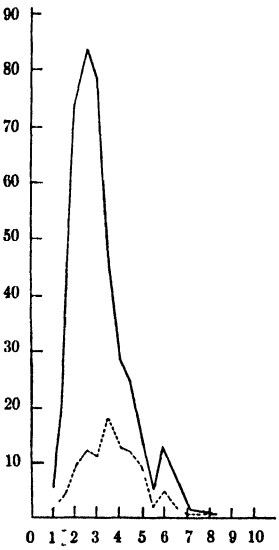


Fig. A. A frequency curve of dimensions of *Boveria teredinidi* plotted from the ratio of the length to the breadth at the oral end. Ratio plotted on the abscissa; the number of the individuals on the ordinate. The solid line indicates the distribution of 400 individuals, the broken line, of 100 of these 400 animals.

Our measurements yielded a wider variation in size than those previously recorded for this species or for *Boveria subcylindrica.* Among the four hundred individuals, the shortest animal recorded was 27 microns long while the longest measured was 173 microns. A correspondingly wide variation occurs in breadth at the oral end, varying from 12 microns to 31 microns. The smallest ratio was 23 microns by 27 microns; the largest, 12 microns by 142.

In figure A, the ratios are shown on the abscissa while the number of individuals having these ratios is indicated on the ordinate.

A second slight peak is produced by thirteen animals having an exact ratio of six, their breadth being 19.2, and their length 115.2, microns. The first peak includes 21 per cent of the animals while the small peak represents only 3 per cent. The latter percentage would have no significance unless it were found to be associated with other distinctive morphological characters such as shape and size of nucleus, length of cilia, and shape of the adoral ciliary zone. Although care was used, no such correlation has been found. We find no significant evidence of two races. Our secondary peak does not represent either of Nelson's (1923) races. This peak includes elongated individuals which are probably approaching the period of binary fission though showing as yet no structural evidence of its approach. It is also possible that the two peaks represent the differential effects of binary fission on the two schizonts.

The broken line of the graph represents the variation of one hundred individuals successively met with on the slide and exhibits both modes found in the above four hundred. This curve falls entirely within the first.

Our preparations agree as to the other specific characters named by Nelson (1910) although we have never observed more than twenty-five food vacuoles. This, however, is a very minor difference. I shall point out other specific characters for *Boveria teredinidi* under the topic morphology.

TECHNIQUE

In our early studies, the gill chamber of the host, *Teredo navalis*, was prepared *in toto* and then the ciliates were teased out. This involved considerable time and it was found difficult to control differentiation in staining. This method, while giving some valuable information, was abandoned in favor of the centrifuge method, the animals being washed out into sea water.

The most successful method found, however, was the employment of the smear method as used in staining amoeba (Kofoid, 1919). No egg albumen was placed on the slide, the mucus present being sufficient to hold the animals in place. The slides were then quickly plunged into the fixing fluid. Comparison of methods showed that the smear method produces no distortion of the animals and it was a much easier way to obtain well differentiated preparations. Centrifuging is less desirable, as some débris and sediment is caught on the cilia and it is more difficult to control staining in bulk.

The following fixing agents were used: Schaudinn's, Bouin's, Zenker's, formalin, osmic acid, and Da Fano's cobalt nitrate-formalin. Among the various stains employed were: Delafield's iron haematoxylin, Mallory's triple connective tissue stain, alum carmine, and silver nitrate toned with gold chloride.

Schaudinn's fluid heated to sixty degrees gave excellent fixation in two minutes. This was followed, as in staining of amoeba, by impregnation of 2 per cent iron alum heated to thirty degrees for two minutes. The time limit for staining with 2 per cent iron haematoxylin, also heated to thirty degrees, was found to be best at from ten to fifteen minutes. Differentiation was carried on in cold 2 per cent iron alum watching the slide under the microscope. Slower staining in weaker solutions produced less acceptable preparations.

Yabroff's application (unpublished work) of Da Fano's cobalt fixative followed by impregnation with silver nitrate and toning with gold chloride, as applied to ciliates, gives excellent results. This method gives beautiful preparations since the silver impregnates the myonemes and neuromotor fibers and brings them out in sharp contrast to other parts of the cell. While the same structures are stained by the iron haematoxylin, the results are not so striking as in the Da Fano method.

Clearing was carried on in xylol, oil of cedar, or a clearing fluid made of equal parts of bergamot, oil of cedar, and phenol. If the organisms were to remain for any length of time in the clearing fluid, as in preparation for imbedding, they were cleared in synthetic oil of wintergreen. Sections were prepared from *Boveria* infiltrated and embedded in gelatin capsules after the manner described by Metcalf (1908).

MORPHOLOGY

Ectoplasmic Structures

Boveria teredinidi is much elongated and subcylindrical in shape during the interphase between fissions. Considerable variation occurs in the body form during the life-history of this organism. In preparation for division, the body first becomes elongated and then noticeably shortened and ovoid; just after division, the oral end is often conspicuously bell-shaped. The anterior end of this species is different in shape from *Boveria subcylindrica* in being rounded rather than pointed (pl. 20, fig. 8). In this it agrees with the variety *Boveria subcylindrica* var. *concharum*.

The *pellicle* covers the entire body with a thin, highly refractive, tenacious film. It is exceedingly flexible, resisting mechanical pressure to a marked degree, forming a protective and retentive covering, as shown by animals which when caught may become twisted and even bend over in the posterior region of the body without injury. The myonemes and their basal granules lie in the outermost region of the ectoplasm directly below the pellicle and when viewed in optical or in transverse section, slight ridges appear on the surface directly over the myonemes.

The *ectoplasm* has no distinct line of demarcation from the *endoplasm* in *Boveria*. In general the ectoplasm is granular; the endoplasm alveolar in structure.

Stevens (1903) describes the ectoplasm as being granular. In favorably stained preparations we find the ectoplasm, with the exception of the area of the peristomal field where the granules are more or less evenly distributed, to be differentiated into longitudinal granular bands or stripes extending longitudinally from the outer posterior adoral fiber, anteriorly narrowing and converging at the aboral end. These alternate with narrow, hyaline stripes. Such granular and clear stripes have been described for *Stentor coerulus* (Johnson, 1893), for *Balantidium* (McDonald, 1922), and for *Tintinnopsis* (Campbell, 1926). No branching or anastomosing of these linear structures has been noted in the species being described.

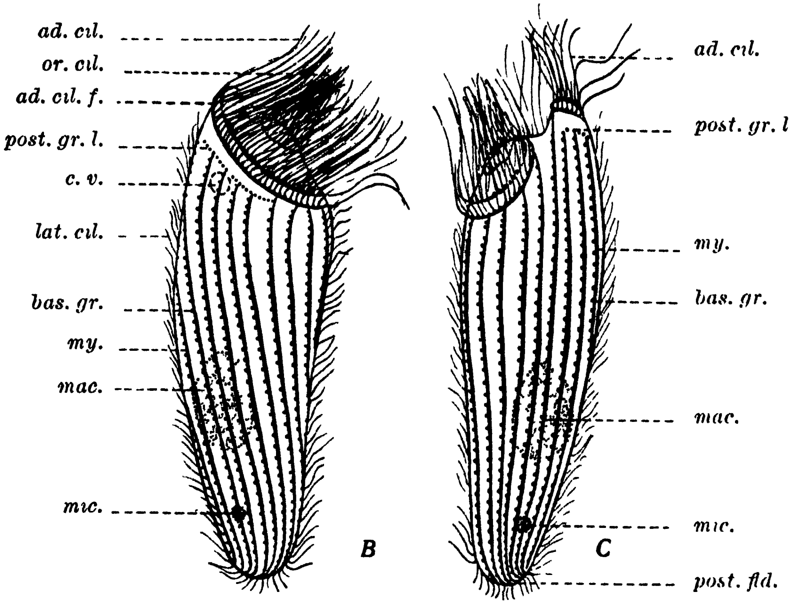


Fig. B. *Boveria teredinidi.* Dorsal side of animal showing oral and adoral ciliary zones. Camera lucida drawing. × 620. Cilia extending from the myonemes, with the exception of those at the sides, are represented only by basal granules.

Fig. C. *Boveria teredinidi.* Ventral side of same animal.

ABBREVIATIONS

ad. cil., adoral cilia; *ad. cil. f.*, adoral ciliary field; *bas. gr.*, basal granule; *c. v.*, contractile vacuole; *lat. cil.*, lateral cilium; *mac.*, macronucleus; *mic.*, micronucleus; *my.*, myonemes; *or. cil.*, oral cilia; *post. fld.*, posterior field; *post. gr. l.*, posterior granular line.

The *cilia* of this heterotrichous protozoan are of two types: (1) body cilia and (2) long, coarse oral and adoral cilia. The somatic cilia are arranged in parallel, or sometimes slightly oblique, longitudinal rows, extending from the posterior adoral ciliary spiral to the posterior end where they converge, leaving a small area devoid of cilia (pl. 20, fig. 8). This bare area on the pellicle may be round and central in position but usually it is oval or oblong in shape and deflected to the dorsal side of the posterior end. This arrangement is due to the separation and converging of the myonemes during division.

Specific characters are to be noted in the number of rows of cilia in the two described species. Stevens (1901) records a variation from twenty to twenty-seven rows in *Boveria subcylindrica* while in *Boveria teredinidi* we find from eighteen to twenty-two. From twenty to twenty-six rows are found in *Boveria labialis* (Ikeda and Ozaki, 1918).

The body cilia are of equal length throughout except that at the aboral end they decrease to one-half this length. They are spaced equidistantly in the rows along the column of the body, but become gradually closer together at the anterior and posterior ends (figs. B–E; pl. 20, figs. 1, 3-6, 8). In movement the vigorous beat of the cilia is directed toward the peristome propelling the animal rapidly forward with the posterior end foremost.

There are two major lines of cilia distinguishable on the truncate peristomal field, (1) the innermost circle of oral cilia (*or. cil.*, figs. B, C) closely set in a single line, arising from basal granules and describing a circle about the cytostome and connecting to the neuromotorium; and (2) the longer adoral cilia (*ad. cil.*, figs. B, C), arising from basal granules forming a double, parallel, dexiotropic spiral peripherally, proximally connecting with the neuromotorium (*neurom.*, fig. D), passing dorsally some distance beyond the neuromotorium, and ending distally in a less extensive leiotropic spiral terminus (*lei. sp.*, figs. D, E; pl. 20, figs. 4, 5, 6). We designate the space between the anterior and posterior adoral fibers (*ant. ad. fib.* and *post. ad. fib.*, fig. D) as the adoral ciliary field (*ad. cil. f.*, figs. B, D). The anterior adoral fiber is within the posterior adoral fiber and marks the outermost margin of the peristomal field.

The oral cilia are slightly shorter than those of the adoral whorls and are more active in feeding. Those on the opposite sides of the mouth slowly open and close curving over the cytostome to prevent the escape of food particles. The cilia of the adoral ciliary field beat synchronously and more rapidly, drawing food particles in from a distance. In order to accomplish this they open wide, then rotate toward the cytostome, closing up with their free ends interwoven.

The length of the cilia in *Boveria teredinidi* is a second point of contrast with *Boveria subcylindrica.* For the latter species Stevens (1901, 1903) gives the following proportions: oral cilia average one-half the length of the body; somatic cilia one-sixth to one-eighth the length of the body. Nelson (1923) states in a very general way that the oral cilia are more than twice the length of the body cilia in *Boveria teredinidi.*

We have found from measurement that in this latter species the oral cilia vary from one-third to one-fifth the body length, while the body cilia are approximately one-fifteenth to one-fiftieth of the body length. During the early stages of division the oral cilia are shortened until they are only about three times the length of the body cilia.

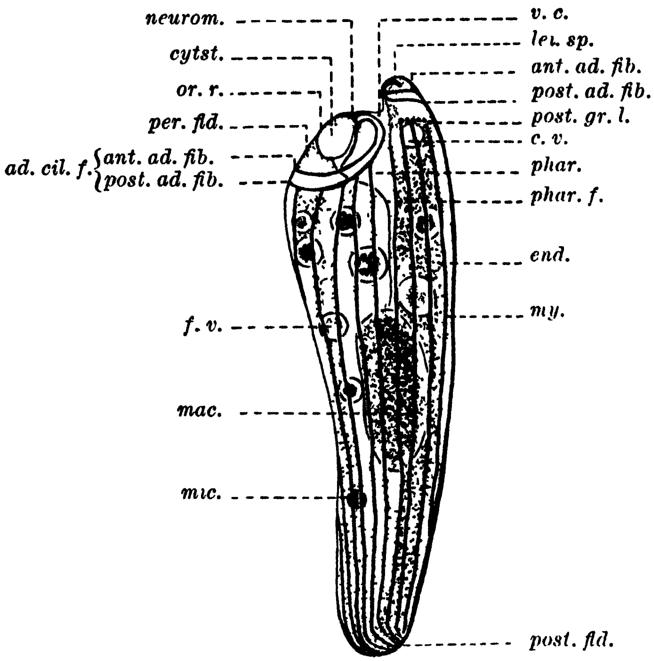


Fig. D. *Boveria teredinidi.* Camera lucida drawing of the animal showing myonemes and oral and adoral fibers in the ectoplasm and deeper structures in the endoplasm. × 620. *ad. cil. f.*, adoral ciliary field; *ant. ad. fib.*, anterior adoral fiber; *c. v.*, contractile vacuole; *cytst.*, cytostome; *end.*, endoplasm; *f. v.*, food vacuole; *lei. sp.*, leiotropic spiral; *mac.*, macronucleus; *mic.*, micronucleus; *my.*, myoneme; *neurom.*, neuromotorium; *or. r.*, oral ring; *per. fld.*, peristomal field; *phar. f.*, pharyngeal fiber; *phar.*, pharynx; *post. ad. f.*, posterior adoral fiber; *post. gran. l.*, posterior granular line; *post. fld.*, posterior field; *v. c.*, ventral cleft.

Myonemes were not observed by Stevens, working with *Boveria subcylindrica* (1901, 1903), and hence she concluded that there were none; nor are they mentioned by Ikeda and Ozaki (1918) or by Nelson (1923). It seems strange that they could have escaped the notice of previous investigators. While they are not visible in living animals they are exceedingly striking in properly stained material. Either iron haematoxylin, the stain used by Stevens and by Ikeda and Ozaki, or silver nitrate toned with gold chloride may be used for demonstrating them.

They are arranged in from eighteen to twenty-two longitudinal rows in the ectoplasm to one side of the hyaline band previously described. At the aboral end they curve inward converging and ending abruptly and unattached at their termination (pl. 20, fig. 8). In the region of the margin of the peristomal field they are connected either by means of granules or of delicate fibers to the posterior adoral fiber in a manner to be described under the neuromotor system. The myonemes appear round in section and are of a fibrous rather than a granular nature.

The large *basal granules* rest directly on the myonemes from which the cilia are extended out through the pellicle.

The *peristomal field,* designated the frontal field by some investigators, is at the oral end of the organism and is truncated into a special area for securing food even at a considerable distance by the strong current produced by the adoral cilia. This area is without cilia. In the interphase between fissions the mouth is slightly elevated from the floor of the field while the outer end of the spire is raised slightly higher than the mouth into a lip, separated from the former by the ventral cleft (*v. c.*, fig. D).

The *cytostome* or mouth in *Boveria* is not a permanent structure and is also variable in size. The mouth and the pharynx can be made out only when the animal is actively feeding. At such times it appears (*cytst.*, fig. D) broad and deep and extending slightly to one side.

Only one *contractile vacuole* is found in each of the species of the genus *Boveria.* In *Boveria teredinidi* it occupies a position dorsally near the outer terminus of the dorsal ciliary zone (*c. v.*, fig. B) being in this respect like *Boveria subcylindrica* var. *concharum.* This vacuole discharges the water-soluble, excreted substances to the outside through a spot in the pellicle which is liquefied at systole (Taylor, 1923). Increase in size is apparently brought about by osmosis as no canals have ever been observed.

ENDOPLASMIC STRUCTURES

The structure of the *endoplasm* is distinctly alveolar, somewhat coarser about the macronucleus than at the ends of the body. The lenticular disc described by Stevens (1901) for *Boveria subcylindrica* is absent as it is also in *Boveria subcylindrica* var. *concharum* (Stevens, 1903) and *Boveria labialis* (Ikeda and Ozaki, 1918).

Food vacuoles are usually found in the anterior end of the animal's body. Each vacuole contains a single leucocyte cell from the host, masses of diatoms, minute Protozoa, or occasionally bacteria. The food vacuoles often continue to persist for some time after the food mass has become liquefied.

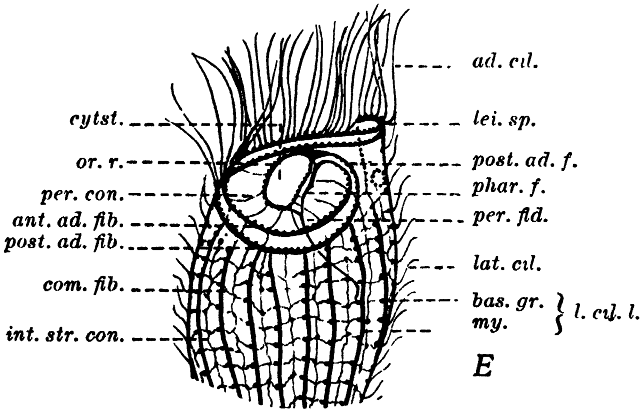
The large, oval *macronucleus* is centrally placed in the interphase, varying from ten to twenty microns in the length of its long axis. When stained with iron haematoxylin, or silver nitrate, it shows a distinct nuclear membrane with the chromatin material scattered over its inner surface and grouped on the linin network (pl. 20, fig. 7). In only one or two preparations have we been able to make out the nucleoli figured by Stevens (1901, 1903). Just previous to and just after division, the macronucleus presents a homogeneous appearance.

The *micronucleus* during the interphase is found between the macronucleus and the posterior end. In shape it is spherical with a diameter of 1.7 microns. In this stage it is surrounded by a clear area in the protoplasm. As division approaches, this clear space disappears and the micronucleus increases from two to three times its original diameter.

NEUROMOTOR APPARATUS

The neuromotor apparatus of *Boveria teredinidi* presents one of the most highly developed and intricate coordinating systems of any ciliate thus far described.

In *Boveria teredinidi* the neuromotor apparatus is made up of eight distinct parts; namely, (1) the motorium (Sharp, 1914) or neuromotorium to which all the motor organelles are directly or indirectly attached by means of fibers; (2) the oral ring which surrounds the cytostome, which begins and ends in the motorium, and which gives rise to a short fiber that joins the pharyngeal fiber; (3) the anterior adoral fiber; (4) the posterior adoral fiber. The latter two fibers give rise to the adoral ciliary lines, making a double parallel dexiotropic spiral about the peristomal field which ends distally to form a short leiotropic loop of half a spiral turn. The anterior adoral fiber is connected by a short strand to the apex of the neuromotorium from which connection it extends close to the left side of the oral ring for approximately one hundred and eighty degrees, where it gives rise to the (5) pharyngeal fiber. The posterior adoral fiber takes its origin directly from the left angle of the neuromotorium distally joining the anterior adoral fiber. The (6) peristomal connectives are exceedingly delicate threads connecting the anterior adoral fiber to the oral ring. (7) The longitudinal contractile myonemes arise at the anterior end, being either directly attached to the posterior adoral fiber by means of fibers or to the posterior granular line. The myonemes pass posteriorly as parallel lines along the column converging at the posterior field. The (8) basal granules of all the cilia, both oral and lateral, rest directly, without fibers of attachment, on their



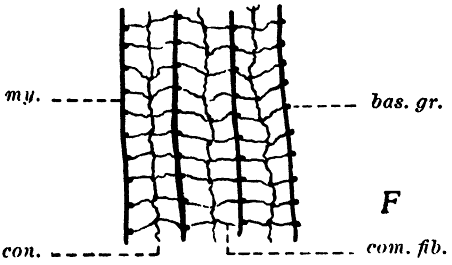


Fig. E. *Boveria teredinidi.* Camera lucida drawing of the anterior half of the animal showing the arrangement and relationship of the neuromotor apparatus. × 825. *ad. cil.*, adoral cilia; *ant. ad. fib.*, anterior adoral fiber; *bas. gr.*, basal granules; *com. fib.*, commissural fiber; *cytst.*, cytostome; *int. str. con.*, interstrial connective; *lat. cil.*, lateral cilium; *lei. sp.*, leiotropic spiral; *l. cil. l.*, longitudinal ciliary line; *my.*, myonemes; *or. r.*, oral ring; *per. con.*, peristomal connectives; *per. fld.*, peristomal field; *phar. f.*, pharyngeal fiber; *post. ad. f.*, posterior adoral fiber.

Fig. F. *Boveria teredinidi.* × 1653. Camera lucida drawing of surface view of column, showing ciliary lines and deeper net work. *bas. gr.*, basal granules; *com. fib.*, commissural fibers; *int. str. con.*, interstrial connectives; *my.*, myonemes.

respective fibers or myonemes. The (9) deeper nerve net consists of interstrial connectives between the myonemes and commissural fibers, uniting transversely the basal granules of the myonemes throughout the entire lateral surface of the column.

The *neuromotorium* (*neurom.*, fig. D; pl. 20, figs. 4, 5, 6), or coordinating center, lies in the ectoplasm on the dorsal side of the cytostome. It stains intensively in iron haematoxylin and in silver nitrate. Its shape may be likened to that of an isosceles triangle. Its apical angle gives rise to the strand which connects the anterior adoral fiber and also receives the oral fiber which takes its origin in the right angle of the neuromotorium. The left angle is the point of origin of the posterior adoral fiber.

The *oral ring* arises from the right angle of the base of the neuromotorium (*or. r.*, figs. D, E; pl. 20, figs. 4, 5, 6) and after surrounding the mouth ends in the apical angle of the neuromotorium. For slightly more than half of its circumference it is a heavy fiber. At this point it becomes slightly thickened, giving rise to a short slender fiber which joins the pharyngeal fiber, and from there on until it reaches the neuromotorium it is a slender fiber dipping slightly deeper into the ectoplasm.

The *adoral ciliary zone* is bounded by two parallel fibers, the anterior adoral fiber and the posterior adoral fiber. Together they curve out from their attachments to the neuromotorium forward on the ventral side following the margin of the peristomal field, passing in a dexiotropic spiral along the dorsal side terminating in a sharp coil in a sinistral direction on the ventral side of the lip, separated from the floor of the peristomal field by the ventral cleft. The large whorl thus described may be compared to a right-hand screw, which, to penetrate, must be turned from left over to right (*ad. cil. f.*, fig. D; pl. 20; figs. 1, 3–6).

The *anterior adoral fiber* (*ant. ad. fib.*, figs. D, E; pl. 20, figs. 1, 4–6) is connected by a narrow strand to the apex of the neuromotorium. From this connection the free end of the fiber continues its course close to the left side of the oral ring in the ectoplasm in the form of a prominent pharyngeal fiber (*phar. f.*, figs. D, E; pl. 20, figs. 4, 5, 6). The outer end of the anterior adoral fiber passes outward in a dexiotropic spiral as previously described.

The *posterior adoral fiber* (*post. ad. f.*, figs. D, E; pl. 20, figs. 1, 3–6) arises directly from the right angle of the neuromotorium, passes out parallel to the anterior adoral fiber and terminates in the leiotropic spiral (*lei. sp.*, figs. D, E; pl. 20, figs. 4, 5, 6).

The *pharyngeal fiber,* as stated before, is a continuation of the anterior adoral fiber receiving near the margin of the peristomal field a short slender fiber which arises from a point on the oral ring on the side opposite the neuromotorium. After receiving this branch, the fiber dips into the endoplasm, following the area occupied by the pharynx during the ingestion of food, and passes posteriorly in a dexiotropic spiral manner around the potential gullet. This striking, long fiber extends very close to the anterior end of the macronucleus where it ends abruptly. There is, however, no connection with the macronucleus.

Within the peristomal field extremely delicate *peristomal connectives* are found to connect, by curved paths, the anterior adoral fiber with the oral ring (*per. con.*, fig. E; pl. 20, figs. 5, 6). They can be made out with certainty only with the highest magnifications. They appear to be direct continuations of the myonemes, although in some cases it has been impossible to trace them to their origin. This may be explained by the fact that following binary fission it is some time before all the nerve network is redifferentiated.

The *longitudinal ciliary lines* are made up of the contractile myonemes and the basal granules. The contractile *myonemes* (*my.*, figs. B–F; pl. 20, figs. 1–6) arise at the anterior end either directly from the posterior adoral fiber or are connected to it by means of delicate fibers running from the posterior granular line. On the ventral side of the ciliate the myonemes to the right of the ventral cleft are all directly attached to the posterior adoral fiber; to the left of the cleft the first myoneme is connected to the distal end of the adoral fiber by means of a fiber. The other myonemes on the ventral side terminate in a row of closely set granules devoid of cilia, which run just below and parallel to the posterior adoral fiber to the middle of the dorsal surface of the animal where the granules join a myoneme directly connected with the posterior adoral fiber. From their orgin, just described, the myonemes pass posteriorly in slightly oblique parallel lines, gradually coming closer together until they converge in a clear area in the ectoplasm designated as the posterior field (*post. fld.*, pl. 20, fig. 8).

The *basal granules* rest directly on their respective fibers or myonemes. The oral ring is closely set with basal granules of cilia as are the anterior and posterior adoral fibers The free end of the anterior adoral fiber beyond its attachment to the neuromotorium is devoid of basal granules. Anteriorly the basal granules of the myonemes are closely set and somewhat smaller. Along the remainder of the myonemes, between the anterior and posterior ends, they are placed quite evenly and equidistant until they gradually become somewhat smaller and closer set as the myonemes approach the posterior field.

Laterally, a *deeper nerve net* intimately connects the area between the myonemes and their basal granules. Delicate, wavy *interstrial connectives* (*int. str. con.*, figs. E, F; pl. 20, figs. 2, 4, 6, 8) run longitudinally midway between the myonemes. Between opposite basal granules delicate, wavy, and irregular *commissural fibers* (*com. fib.*, figs. E, F; pl. 20, fig. 8) form the transverse connectives. At their points of intersection with the interstrial connectives are small irregular-shaped granules lying at varying levels in the ectoplasm.

DISCUSSION

The delicacy of parts, the structure, position, and complex integration of fibers in the system just described for *Boveria teredinidi,* seem sufficient evidence to disprove any hypothesis of a supporting function. The extreme complication and association of parts makes an efficient apparatus over which sensory impulses could be sent to the directing center and over which motor impulses might be sent to the organelles of locomotion.

The oral ring, the anterior and posterior adoral fibers are all directly connected with the neuromotorium forming complete pathways for the conduction of nervous impulses in the coordination of these organelles. The long coarse cilia originating from basal granules resting directly on these fibers might serve not only as sensory organelles, but at the same time also for the conduction of motor impulses and for movement.

The lateral body mechanism, consisting of the longitudinal ciliary lines and the deeper nerve net, is in turn directly connected to the posterior adoral fiber, thus securing coordination in action of all the cilia of this system. The myonemes are contractile in function and are also conductors of nervous impulses transmitted by the lateral cilia to their basal granules and thence to the myonemes and neuromotorium. The lateral cilia, while chiefly motor in function, are also sensory and conductive of impulses. The deeper network completes the very elaborate coordinating system by providing transverse as well as longitudinal pathways.

The neuromotor systems of five other ciliates, *Diplodinium ecaudatum* (Sharp, 1914), *Euplotes patella* (Yocum, 1918), *Balantidium coli* and *suis* (McDonald, 1922), *Paramecium* (Rees, 1922) and *Tintinnopsis* (Campbell, 1926) have been described.

Of these five examples of neuromotor systems, four are alike in that neuromotoriums have been found. Rees does not figure a motorium, but refers to a neuromotor center which he believes corresponds to the motorium of other ciliates. In all, the neuromotorium lies in the ectoplasm near the cytostome. By means of fibers, it is connected with some or with all of the motor organelles of the animal. *Boveria teredinidi* follows the general plan of a neuromotorium near the mouth with fibers passing from it to the various motor parts. The deeper peripheral net here described provides a complete integrating mechanism of the elaborate type.

The oral ring of *Boveria* is perhaps analogous to the circumoesophageal ring of *Diplodinum;* the pharyngeal fiber corresponding to the oesophageal fibers in the wall of the oesophagus of *Diplodinium;* here the comparison ends, since there are no lateral cilia present in *Diplodinum.*

Evidence for the neural function of the neuromotor system in the past has been of two types, morphological and experimental. Physiological evidence is to be had in the behavior and response to stimulation, contraction, and movements of these ciliates. The morphological position and delicacy of the nerve net supports the hypothesis of a neural function. The experimental evidence is furnished in the delicate microdissection experiments performed by Taylor (1920) on *Euplotes patella.*

Rees (1922) did not procure as convincing results in his microinjection of vital dyes and *intra vitam* staining with methyline blue.

All investigators in the study of neuromotor systems have found that it takes in some degree the acid fuchsin in Mallory's triple connective tissue stain, which suggests that the fibers resemble chemically the nerve fibers of the Metazoa.

A biochemical evidence is suggested from the similarity of the stains which demonstrate the neuromotor system of *Boveria* and that of the Metazoa. Identically the same structures in *Boveria* are brought out by the use of Da Fano's silver-gold method as those obtained from iron-alum haematoxylin. The former method is the principal one used for demonstrating the nerve cells of the Metazoa. Here again the conclusion is suggested that possibly the neuromotor apparatus of the Protozoa is made of material chemically similar to that in the nervous tissues of the Metazoa.

SUMMARY

Boveria teredinidi was described as a new species by Nelson (1923). This species may be further characterized by an adoral spiral which forms a dexiotropic whorl of one and a quarter turns continued as a short leiotropic whorl distally; eighteen to twenty-two rows of lateral cilia attached by basal granules to longitudinal myonemes; oral and adoral cilia from one-third to one-fifth the body length.

Measurements, made during the present study, do not show the presence of two distinct types for this species as described by Nelson (1923). The approach of binary fission or possibly the differential results of binary fission itself give rise to an elongated form.

A consideration of the sum total of all the characteristics of *Boveria* shows a closer relationship to the Heterotrichida, to which it is removed from the Holotrichida, where it was placed by Calkins (1926).

A new family, Boveridae fam. nov., is proposed for this group of ciliates under the Heterotrichida.

The neuromotor system consists of a neuromotorium from which fibers pass to the different regions of contractile activity. These fibers consist of (1) an oral ring surrounding the cytostome, which gives rise to a branch of the pharyngeal fiber; (2) a posterior adoral fiber running from the neuromotorium connecting the posterior adoral cilia; (3) a second fiber, the anterior adoral fiber, attached to the neuromotorium by a strang, giving rise to the anterior adoral cilia and at its distal end joining the posterior adoral fiber; (4) the pharyngeal fiber. The lateral coordinating system consists of longitudinal myonemes with their basal granules and cilia and a deeper peripheral network of interstrial connectives and commissural fibers. The entire lateral apparatus is directly connected to the posterior adoral fiber by means of connectives and fibers.

The substance of the neuromotor apparatus of the Protozoa seems to be similar to the nerve tissues of the Metazoa, since both are demonstrated by Da Fano's silver-gold stain.

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San Francisco, California.

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EXPLANATION OF PLATE

PLATE 20

Boveria teredinidi Nelson from *Teredo navalis*

Figs. 1, 3–8 were fixed in Da Fano cobalt nitrate, and stained with silver itrate.

Fig. 2 was fixed in Schaudinn and stained with iron-alum haematoxylin.

Figs. 1, 3, 5, 6, 8, × 745.

Figs. 2, 4, 7, × 1490; finer structures have been worked out with × 3200. omatic ciliation, except at sides, is indicated by basal granules only. Cilia f adoral zone shown only at back of ciliate.

Fig. 1. Dorsal side of body of ciliate showing the arrangement of the ngitudinal ciliary lines and the oral and adoral spiral.

Fig. 2. Surface view of portion of ectoplasm below the pellicle, showing e myonemes, basal granules, interstrial connectives, and commissural fibers.

Fig. 3. Ventral view of the same animal shown in fig. 1.

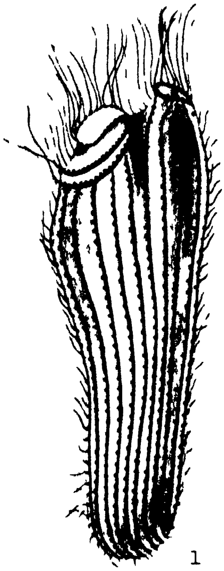
Fig. 4. Ventral view of ciliate showing in detail the neuromotor system.

Fig. 5. Right side of *Boveria teredinidi*, showing oral and adoral fibers and iliary lines; also food vacuoles, macronucleus, and micronucleus in the endoplasm.

Fig. 6. Anterior end of an animal, showing the arrangement and relation- hip of the neuromotor apparatus. Somatic ciliation indicated by basal ranules.

Fig. 7. Nucleus in interphase showing chromatin granules on nuclear mem- rane, the linin network, and the central region of chromatin granules.

Fig. 8. Posterior end of *Boveria teredinidi*, showing myonemes with their asal granules converging at the posterior field. Note deeper network between yonemes.



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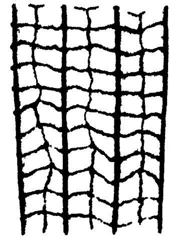

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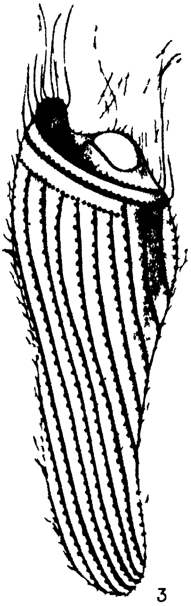

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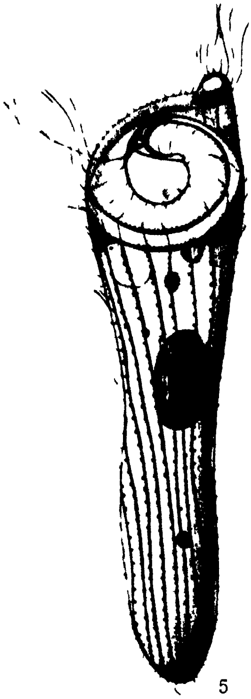

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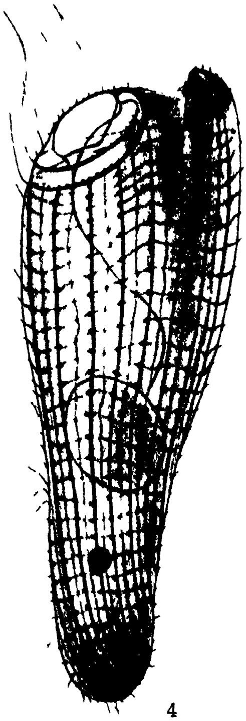

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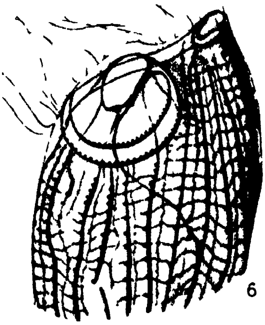

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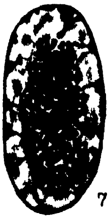

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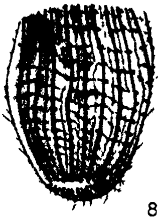

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STUDIES ON THE MARINE CILIATE FAVELLA (JÖRGENSEN), WITH SPECIAL REGARD TO THE NEUROMOTOR APPARATUS AND ITS RÔLE IN THE FORMATION OF THE LORICA

BY

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INTRODUCTION

Prominent among the marine plankton collected in a fine silk net are various Tintinnoina. Catches made in San Francisco Bay and at La Jolla during the year 1925 showed, at various times, large numbers of the genus *Favella* (Jörgensen). The technical methods employed in preparing the specimens for study were similar to those described in an earlier paper (Campbell, 1926).

To Dr. C. A. Kofoid, under whose direction this work was undertaken, and to Dr. T. Wayland Vaughan, of the Scripps Institution of Oceanography, I owe thanks for many courtesies.

SYSTEMATIC POSITION

The genus *Favella* has been recently erected to include certain forms of Tintinnoina previously allocated to *Cyttarocylis* and forming a distinct section of the latter. Jörgensen (1924) as a result of his collections in the Mediterranean concluded that these forms formed a homogeneous series and might be profitably distinguished as a genus. He diagnoses *Favella* as having a typical "*Cyttarocylis* structure,'' without a collar, save in *F. ehrenbergi* and *F. azorica*, and without coiled structure. For this genus he selects as the type, *Favella serrata* (Möbius). ''Here,'' says Jörgensen, ''belong the rest of the old-established genus *Cyttarocylis,* after the separation of *Xystonella, Xystonellopsis, Coxliella* and the genus *Cyttarocylis* proper'' (1924, p. 25). In the genus *Favella* belong, according to Jörgensen (1924), not only the type-species, *F. serrata* (Möbius), but in addition *F. ehrenbergi* (Clap. and Lachm.), *F. azorica* (Cleve), *F. adriatica* (Imhof), *F. markusovzkyi* (v. Daday), *F. aciculifera* Jörgensen, and *F. fistulicauda* Jörgensen.

In our investigation *F. serrata* is treated in some detail and unless otherwise stated it is this form that is discussed.

MORPHOLOGY

The form of the *lorica* (pl. 21, fig. 1) is that of a much elongated bell with a short, slender, pointed handle (*pd.,* fig. B, 3). It opens at its widest part to form the oral aperture and narrows somewhat posteriorly at the fundus where the bowl ends. The *pedicel* (fig. A, 1, 2, 4, 7) is seldom straight and generally curves to one side or the other. It is sometimes provided with a slight knob at the tip. The bowl (fig. B, 3) of the lorica is usually straight-sided but somewhat below its oral margin it may become locally somewhat distended with the result that the oral margin is slightly narrower than is this widest part of the bowl. In *F. ehrenbergi* the sides are rounded evenly and regularly (fig. A, 6). The *lumen* (*lu.,* fig. B, 3) of the bowl is capacious and the inner surface of the bowl is smooth. The lumen extends into the pedicel nearly to its tip, which is closed (*lu. ped.,* fig. B, 3). *F. ehrenbergi* does not have a hollow pedicel (fig. A, 6). The contour of the lumen (fig. A, 3, 6) follows closely that of the bowl except that the wall becomes thinned out orally just below the lip.

The *oral margin* is serrate or denticulate. The *denticles* (pl. 21, fig. 1), arising from broad bases, extend upward in a regularly spaced series in the thin oral margin of the lorica. These denticles are acute, short, and erect, and seldom higher than three microns. They are not undulations but rather sharp. There are sixty to seventy-five of them. Their function is difficult to make out. There is some correlation between the number of these denticles and the number of ciliary lines on the cytosome.

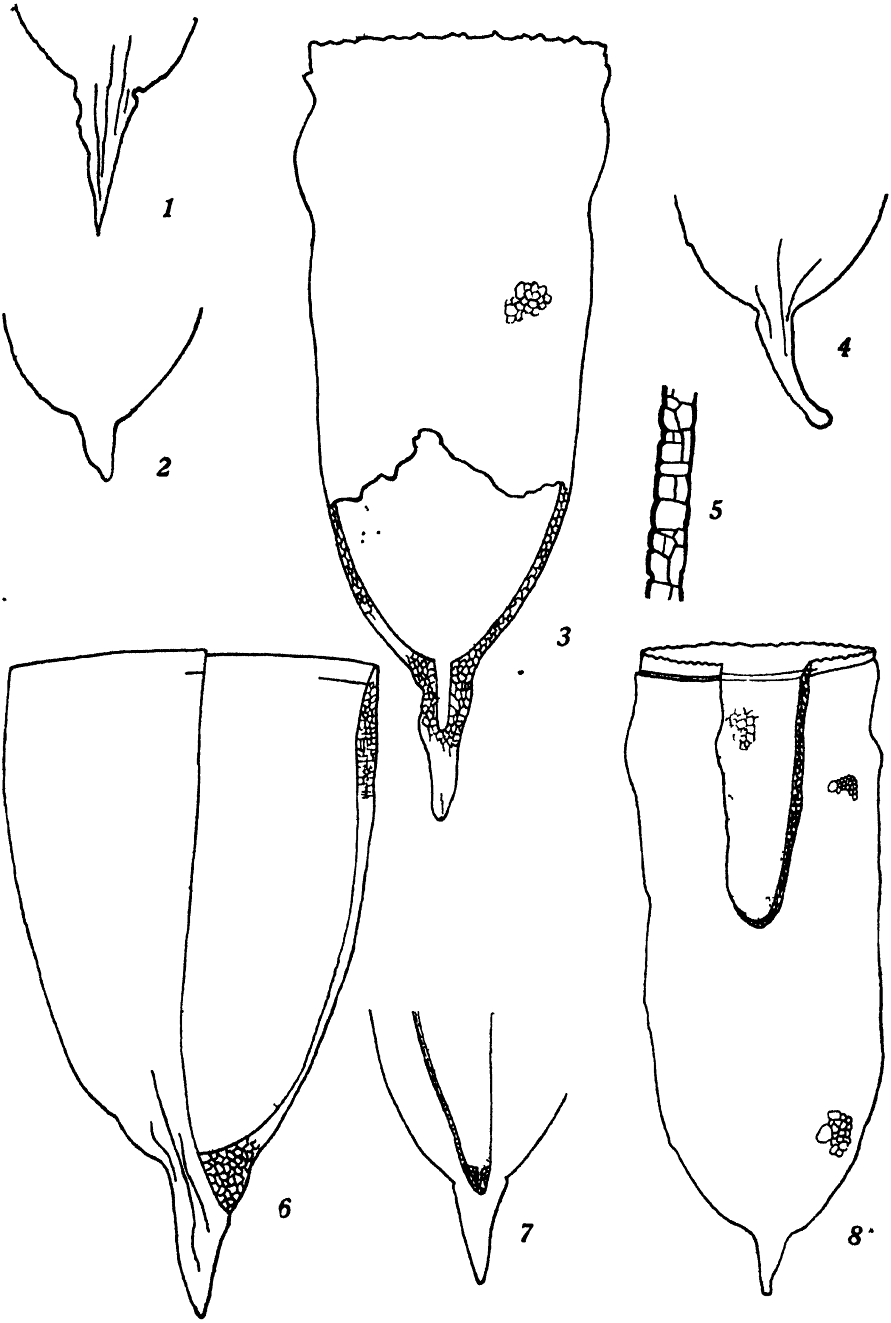

Fig. A. Camera lucida drawings of *Favella*. 1. Pedicel of *F. serrata*. 2. Pedicel of *F. ehrenbergi*. 3. Lorica of *F. serrata*. 4. Pedicel of *F. serrata*. 5. Wall section of *F. serrata* to show structure, greatly enlarged. 6. Lorica of *F. ehrenbergi*. 7. Pedicel of *F. serrata*. 8. Lorica of *F. serrata*. × 422.

The *wall* of the lorica is distinctively alveolar (fig. C, 2). The alveoles of the wall vary in dimensions from those of about one micron across to larger ones of two or four microns. Often, in the upper part of the lorica near the oral margin, there are large conspicuous, pellucid lacunae. These appear to owe their origin to a differential colloidal aggregation, possibly brought about when the lorica is first formed by some feature of food or excreta. The alveolar structure, which is characteristic of the cyttarocylids and widespread in other Tintinnoina, is formed from layers of secreted colloidal matter of at least two sorts, one possibly forming the wall of the alveole, and the other the contents. The visible walls of the prismatic elements (Kofoid, 1905) are apparently the outer, closely compressed walls of the crowded and crushed substance-filled alveoles described. This is clearly shown by Entz, Jr. (1909, pl. 20, figs. 42–43). The subregular prisms (pl. 21, fig. 4) which make up the wall are so placed that their ends form the inner and outer surfaces of the lorica. Their sides form the hexagonal or rounded mesh of alveoles which is designated as the 'secondary structure' (Brandt, 1907). Their ends may form the inner and outer lamelles as suggested by Kofoid (1905). A finer alveolar structure (pl. 21, fig. 4) of 'primary alveoles' may be detected on the inner and outer surfaces, within the coarser secondary ones (fig. C, 2). These two types of alveoles probably owe their origin to differential factors of colloidal aggregation. The outer ends of the peripheral alveoles of the reticulum are hemispherical or irregular and lend a roughened appearance to the wall in addition to the folds or pleats. The whole outer surface of the lorica is unevenly rugose. The short, longitudinal pleats are developed to the greatest extent near the pedicel. The inner face of the wall is smooth and the coarse '*Cyttarocylis*-structure' is evident on the outer face only. The alveoles which make up the wall are found between the lamelles, in several layers. *Favella serrata* never shows annulation, although the localized bulging below the rim simulates a tendency to the formation of an annulus.

The alveolar structure is pronounced in the aboral half of the lorica where the alveoles are larger and in many layers and is less apparent above. The alveoles are usually in one to three layers and may be in four or even five layers aborally, especially in *F. ehrenbergi*.

In its measurements *F. serrata* falls into what Brandt (1907) calls the "large tintinnids." My measurements of individuals collected off-shore in California range between 220 and 260 microns in length.

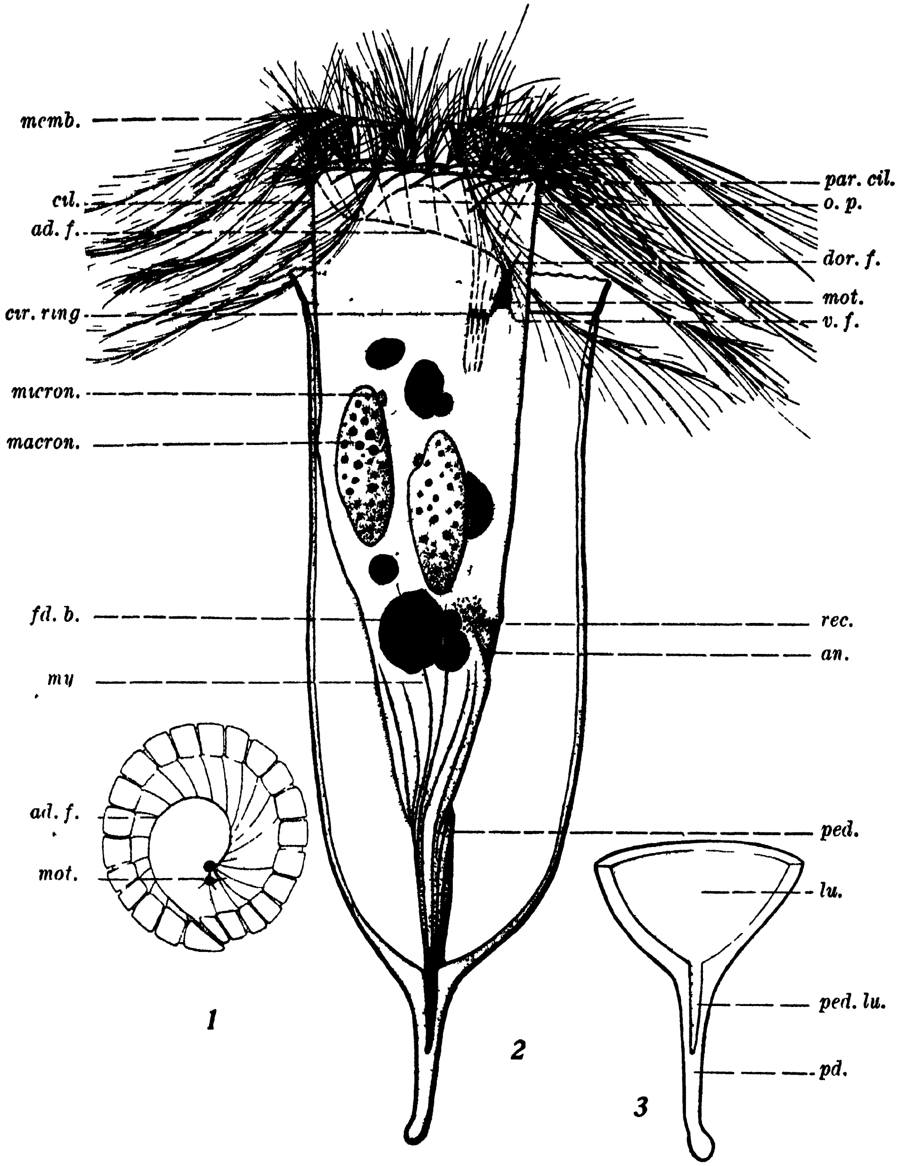

Fig. B. Semidiagram of *Favella*. 1. Cytosome from above, showing adoral fiber, *ad. f.*; motorium, *mot.* 2. Cytosome, showing adoral fiber, *ad. f.*; anus, *an.*; cilia, *cil.*; circumoesophageal ring, *cir. ring*; dorsal fiber, *dor. f.*; food body, *fd. b.*; macronucleus, *macron.*; membranelles, *memb.*; micronucleus, *micron.*; myonemes, *my.*; paroral cilia, *par. cil.*; peduncle, *ped.*; oral plug, *o. p.*; rectum, *rec.*; ventral fiber, *v. f.* 3. Posterior end of lorica, lumen, *lu.*; pedicel, *pd.*; pedicel lumen, *ped. lu.* × 525.

They average about 225 microns in total length. They are 70 to 82 microns across the oral aperture so that they form a length to breadth ratio of 2.5–3.2 oral diameters in length, averaging 2.8. This ratio appears to vary considerably from collection to collection and may be indicative of the widespread possibilities of growth, reproduction, secretion, and flotation factors. The length of the pedicel also varies considerably in any collection (fig. A, 1, 2, 4, 7). Some forms have a short stumpy pedicel of 17 microns in length, while others have one of 50 microns. The pedicel may then vary between 1:1.8 to 1:5.5 the oral diameter, or 1:4.8 to 1:11.8 total length. The pedicel is 14 to 18 microns across at its origin and is usually conical in shape. It is ribbed with longitudinal folds, especially in *F. ehrenbergi* and *F. serrata* (fig. A, 4, 6) and may be knobbed at the tip. This pleating and knobbing may be a structural evidence of the manner of formation of the lorica through building activities.

In the *cytosome* of *Favella serrata* several major regions may be distinguished. These are the peduncle, body, nuclear system, and finally the neuromotor system. These will be treated in order. The animals are somewhat trumpet-shaped, roughly resembling the shape of the lorica in which they dwell. There is a flattened oral end surmounted by a spiral crown of membranelles.

The *peduncle* (*ped.*, fig. B, 2) is the narrow conical part which extends posteriorly from the main part and fastens the animal to the lorica. At the point of junction with the lorica it expands slightly and ends in the canal of the pedicel. In *F. ehrenbergi* there is no canal so that the peduncle is fastened in the fundus. The peduncle is sometimes parted into lateral branches, which may freely anastomose with each other, and form attachments to the wall. The peduncle is not homogeneous in make-up. Within the living animal tiny granules are visible, which upon fixation and staining are often deeply colored, as though this might be a region of secretory activity. The animal may attach itself to the lorica either by secretion or by suction; possibly both are involved.

In the somewhat thickened granular periphery of the peduncle are pronounced fiber-like *myonemes* (*my.*, fig. B, 2) which ascend vertically about halfway up the length of the body. These myonemes are stouter aborally and thin out orally. They are granular in the fixed and stained animal and thread-like in the living organism. They are unlike those of *Tintinnopsis* (Campbell, 1926) in that they do not seem to extend up to the margin of the frontal field. That they continue along the ciliary lines and join with the adoral motor apparatus is, however, probable, although difficult to trace. The myonemes are similar in number to the membranelles, that is, there are about twenty-four. The peduncle is ciliated only on its upper two-thirds.

The trumpet-shaped body, or *cytosome* proper, surmounts the narrow and rather distinct peduncle already described. It constitutes the greater volume of the organism; in it lie the trophic mass and the nuclei. The motor and feeding organs crown its flattened oral frontal field. As is the peduncle, so also the body is colorless, except as colored food-bodies occupy large vacuoles within it. The body is 50 to 70 microns in diameter and 200 to 265 microns in length, when fully extended.

There is no thick peristome collar enclosing the frontal field as in *Tintinnopsis* (Campbell, 1926); instead the cytosome continues and narrows somewhat as it turns inward to surround the margin of the frontal field, forming, as it does so, a *suboral constriction*. The margin of the frontal field is lobate or undulating, without tentaculoids.

An examination of the *frontal field* from above (fig. B, 1) reveals the most complex and integrated portion of the whole organism. This frontal field is subcircular, concave, except for the projecting oral plug, and occupies the whole oral end of the animal. It spirals ventrally downward toward the deepest part where the excentrically located *infundibulum* leads to the mouth below. Its surface is striated, regularly alternating bands of clear and darker substance forming a spiral which enters the infundibulum. Dorsolaterally arises a highly mobile prominence, or *oral plug* (*o. p.*, fig. B, 2) like that of *Tintinnopsis* (Campbell, 1926) and characteristic of the tintinnids as a whole. While the frontal field may be said to be subcircular and has been so regarded for a long time, a more careful examination demonstrates that it forms a spiral like that of *Stentor* (Johnson, 1893) and other heterotrichous ciliates. The direction of the spiral is left-handed or leiotropic viewed from below. Between the distal and proximal ends of the spiral there is a thin, scarcely marked, but distinctly evident cleft upon the margin of the frontal field. The distal end of the spiral tapers as it ends at the cleft and overlaps the proximal part for a little distance. Thus the frontal field becomes a distinct series of organs of the animal and is not to be regarded as a hypostome. The frontal field is always transverse to the main axis of the organism unlike *Tintinnopsis* (Campbell, 1926) or *Amphorella* (Fauré-Fremiet, 1924), in which its face is usually at a considerable angle to that axis.

A prominent *anus* or *cytopyge* (*an.*, fig. B, 2) is located in the lower part of the body, below the mouth and on the same side, at a point just above the oral origin of the peduncle. There is a raised slit in the cuticle which marks its position. When fully open the *rectum* (*rec.*, fig. B, 2) extends as a short canal into the endoplasm. There are no *contractile vacuoles* in these forms and indeed I have been unable to find any in the marine Tintinnoina I have examined. Brandt (1907) lists a series of marine forms with contractile vacuoles.

The *infundibulum* descends from the peristomal field, plunging into the slightly spirally disposed gullet, after leaving the mouth. There are no structural changes associated with this deep funnel-shaped depression as it leaves the peristome and descends the gullet. The infundibulum may occupy one-fourth to three-eighths of the breadth of the peristome. Its walls are partly lined by several regularly spaced membranelles.

The *gullet* leads posteriorly from the infundibulum after leaving the mouth. It forms a curved tube, the convex side of which is directed toward the outer ectoplasm and the concave side toward the oral plug. It is one-fourth the length of the body and when fully expanded it may be fifteen microns in diameter at the entrance. It is lined by pellicle. Extending down its length are three rows of short cilia which arise from the membranelles which enter the infundibulum. At the anterior end of the gullet is the *cytostome* or mouth.

The surface of the body and the peduncle is covered with a *pellicle* slightly more than a micron across where it is widest. That part of the pellicle which covers the frontal field and bears the first shock of surrounding influences, is somewhat thicker, as is the part about the slit-like cytopyge. Its surface is unmarked. It is flexible, retentive, and protective in function, and assists in the rapid and extreme changes in form which take place in this very mobile organism.

A distinctly differentiated and sharply limited *ectoplasm* does not distinguish any tintinnid (Entz, Jr., 1909) and no line of structural demarcation is found in *F. serrata.* The large alveoles of the endoplasm merge insensibly with the smaller, denser granules of the periphery.

The *endoplasm* makes up the greater part of the organism. In it digestion and other metabolic processes proceed. The cytoplasm which makes up the central core of the body is highly vacuolated, while that of the oral plug is denser. The endoplasm is crowded with food vacuoles. Most of these are found just above the peduncle which suggests that this region, near the cytopyge, is a food-storing one. The whole endoplasm is highly fluid and readily responds to changes which deform the body; there is little evidence of active streaming in it.

There are two *macronuclei* (*macron.*, fig. B, 2) and two *micronuclei* (*micron.*, fig. B, 2), one of the latter associated with each of the former, and set deeply in the endoplasm, one macronucleus behind the other, behind the gullet and somewhat lower. In the living animal the macronuclei appear as large gray masses and are clearly brought out by aceto-carmine and methyl-green. They readily shift in position, especially at the onset of division and may then occupy a position near the ventral side below the gullet, one macronucleus on either side of the anlage of the new frontal field. In form the macronuclei are somewhat like short sausages. The ends may be strung out irregularly and become ragged in some individuals. The finer structure of the macronuclei is much like that of *Tintinnopsis* (Campbell, 1926). The micronuclei are globular and generally located in depressions in the macronuclei, moving out into the endoplasm only during division. The macronucleus is 8 to 15 microns in width, 35 to 55 microns in length. The micronucleus is 2 to 3 microns across. Fauré-Fremiet (1924) claims that there is but a single micronucleus located about halfway between the two macronuclei in *F. serrata.* Two are uniformly present in my material.

NEUROMOTOR APPARATUS

The neuromotor apparatus includes all the motor elements of the organism and a connected fiber system which centers in the neuromotorium. It makes up a remarkable receptor-effector system which is concerned with the activities of the organism.

There are about twenty-four *adoral membranelles* (*memb.*, fig. B, 2). I have counted as few as twenty and as many as twenty-six in some cases. Each of the membranelles is about forty-five microns in length from the distal tip to the origin below. They form a leiotropic spiral crown which follows the course of the margin of the frontal field. At the base they connect with each other and with the neuromotorium by a fiber. Three of the proximal membranelles enter the infundibulum and become modified to form the oral membranes, which are made up of short cilia. The membranelles are blade-like and flat. The outermost row of cilia is thick and stiff. Against these the distal ends of the remainder of the cilia may beat rather freely. At the base of the membranelle are three large basal bodies. The cilia which compose the membranelles are long. The function of the membranelles is not only to serve the animal in feeding and in locomotion but also to function in the building of the lorica during periods of division.

The *cilia* which make up the somatic coating are differentiated into longer *paroral cilia* (*par. cil.*, fig. B, 2) which occupy the suboral margin of the frontal field, and the short, barely visible *somatic cilia* which cover the surface of the cytosome (*cil.*, fig. B, 2). All these cilia are arranged in vertical rows. The paroral cilia may be three to five microns in length. There are about seventy of these rows of cilia and they are never differentiated locally into a lateral field as in *Tintinnopsis* (Campbell, 1926). Since this number of ciliary lines corresponds closely to the number of denticles which surround the mouth of the lorica, there is a suggestion of correlation between them. The longer cilia which are present suborally may serve as moulding organs and arrange the substance which makes up the lorica in this region into the characteristic denticles. In *F. serrata* these rows may build up denticles, whereas in *F. ehrenbergi* they may smooth down the lip.

The *fiber system,* which is the conducting and integrating system, lies in association with the organs just described. It comprises the (*a*) neuromotorium, (*b*) the adoral fiber, (*c*) the circumoesophageal ring, and (*d*) the ventral and dorsal fibers which arise from the motorium.

The *neuromotorium* (*mot.*, fig. B, 1, 2) is the large central massive spindle-shaped body toward which all the fibers in the system, by fusion or direct course, ultimately converge. It is located in the ventral ectoplasmic wall at a point below the middle of the gullet. From its aboral end the two *ventral fibers* (*v. f.*, fig. B, 2) arise and from its adoral end springs the *adoral fiber* (*ad. f.*, fig. B, 2). The motorium is rather small and is differentiated, with some difficulty, in whole mounts stained in iron-haematoxylin. The adoral fiber extends from the neuromotorium around the frontal field and below its margin (*ad. f.*, fig. B, 1). It connects each of the membranelles and forms the principal neuromotor trunk. In addition to the adoral fiber the dorsal fiber appears to connect also with the striations of the oral plug. It was probably this fiber and the adoral fiber which Entz, Jr. (1909) described in *F. ehrenbergi* but which he did not connect with a central neuromotorium, which he did not detect and hence was led to conclude that the function of contractility and conduction resided in the ectoplasm. The ventral fibers appear to vanish freely in the endoplasm, passing downward. The *circumoesophageal ring* (*cir. ring.*, fig. B, 2), which is one of the most interesting parts of the system, surrounds the gullet below the midpoint and connects with the neuromotorium by a fiber. Fibrils extend from it below about the gullet.

The oral and pedicel regions are the areas of greatest morphological differentiation, and the oral region is the seat of the neuromotorium, which is the directing center. The motile organs, the membranelles, and cilia are located on or near the oral end and in the closest association with the areas of greatest modification in the loricas of all or nearly all the Tintinnoina (cf. Brandt, 1906) at binary fission. These facts lead us to suggest that the lorica forms a sound basis for systematic studies, even in the absence of the animal, for it is a biochemical product formed in a characteristic manner for each species and based both on the products and also upon the specific behavior of the species.

ECOLOGY

Favella serrata appears to be largely neritic, but it is also found under oceanic conditions, being recorded in the North Sea by Brandt (1907). In general it is widely distributed in temperate and subtropical seas. It was abundant in the waters of San Francisco Bay in August, 1925, and was found in collections made at La Jolla during January of the same year. In all these collections which I made living animals were present. *Favella serrata* is an epiplanktont. Some collections yield many individuals while others taken a few days later may yield only a few.

The food of *Favella serrata* consists of bacteria, small pelagic diatoms and dinoflagellates, mostly common species, and more rarely, radiolarians, silicoflagellates, and some chrysomonads, whose yellow chromatophores add to the coloring matter of the cytosome. Entz, Jr. (1909) reports the using of coccolithophores and small nonloricate ciliates. These food bodies lie in vacuoles (*fd. b.*, fig. B, 2), where they persist for a long time. There is no indication that these are selected in a particular manner. One may find dinoflagellates, diatoms, and other food matter in the same individual.

Favella serrata moves with the aboral end foremost, or it may reverse and move with the adoral end foremost. Locomotion is less darting than in *Tintinnopsis* (*Campbell*, 1926), having more the course of a smoothly moving *Paramecium*. *Favella serrata* does not adhere to solid objects, but is gregarious like *Tintinnopsis*. When disturbed it may desert its lorica and swim free. The animal rotates about its long axis as it moves forward.

Turning to the membranelles we find that these organs move in a harmonic, rhythmic, and pulsating manner, which accomplishes the work of locomotion and feeding, and, at certain periods, shell-building. When at rest, isolated, single membranelles may act independently. In locomotion the waves of contraction move distally on the membranelles from one to the next rhythmically. Oral membranelles, found in the mouth, are concerned with feeding and the organization of small food objects into boluses. The animal bores its way through the medium in which it lives in the manner described rather carefully by Entz, Jr. (1909). Mucus, strung out behind the moving animal, may be drawn up during resting periods and organized into food masses, together with the adhering food matter gathered by chance in this way.

There is some evidence that flotation is a function of the lorica in the Tintinnoina. Not only the great specific surface but the lightened structure is of importance to flotation. The alveolar structure whereby the hard wall probably encloses less dense substance enables the greatest surface to be attained with the least weight. This alveolar structure is greatly developed in *Favella* and similar Tintinnoina and may be of advantage in flotation. The long pedicel at the aboral end of the lorica may act as suggested by Brandt (1896) as a counterweight to the swimming animal, or may serve to increase the size of projection (Oswald, 1903*a*, *b*) and maintain the center of gravity so that a horizontal position is retained. Rapid sinking is prevented by the enlarged specific surface.

DIVISION IN SPECIAL RELATION TO THE FORMATION OF THE LORICA

The *cytosomal changes* of *Favella serrata,* in division, are in principle not unlike those of *Tintinnopsis nucula* (Campbell, 1926). The cytosome elongates, and, as this continues, the newly forming frontal field on the side of the cytosome appears in increasing detail. As in *Tintinnopsis* the newly formed field after arising as a clear line curls upon itself and finally forms a spiral upon the ventral side of the parent cytosome. As the spiral grows and winds, the body continues to elongate. A fully formed, although very small functional frontal field, provided with short membranelles, appears in this position long before there are nuclear changes. Other stages of the process of cytoplasmic division are similar to *Tintinnopsis* (Campbell, 1926) and are illustrated in *F. serrata* (fig. C, 1, 5, 6, 7).

One cannot help being struck with the great part played by the cytoplasm in differentiation during division. The formation of the daughter peristome precedes all visible morphological changes in the nuclei of both *Tintinnopsis* (Campbell, 1926) and *Favella* (fig. C, 1). There is a daughter peristome formed with beating membranelles for some time before division changes are apparent in the nuclei (fig. C, 1). This fact leads us to believe that the neuromotor system, and the basal granules of the membranelles are derived from a cytoplasmic source and not, as maintained by Entz, Jr. (1909). from granules discharged by the nuclei. We suggest that the granules seen by Entz, Jr. (1909, pl. 16, figs. 8–10) are capable of another explanation, and cite in support of this suggestion the evidence from *Vorticella,* in which the aboral ciliary apparatus arises *de novo* and suddenly, in response to freedom from the stalk, either when freed naturally as in division or experimentally by cutting (Runyan and Torrey, 1914). This evidence is contrary to that expected from a nuclear origin for the basal bodies and the neuromotor system.

Nuclear changes in *Favella serrata* during division occur sometimes after the new frontal field has appeared upon the surface of the column. They are very similar to those of *Tintinnopsis* (Campbell, 1926). The macronuclei rotate into position one beside the other about the anlage and after some time elongate, and the chromatin materials within become strand-like (pl. 21, fig. 3). The reconstruction band in the macronucleus appears probably as the solution of contents in this area proceeds. Following this the daughter nuclei separate and then reassemble. The micronuclei divide mitotically, forming a large number of chromosomes. They swell up to about five microns in the prophase. There is never any indication of conjugation or condensation as described by Laackmann (1906) or Entz, Jr. (1909), either of the macronuclei or micronuclei. Each always retains its identity throughout all phases of division and interkinesis. There is no evidence that chromidia give rise to nuclei, as was thought by Entz, Jr. (1909). The nuclei may be parasitized by *Karyoclastis* (fig. C, 4), or the cytosome by *Duboscquella* (Chatton, 1920) (fig. C, 3). Monomacronucleate individuals (pl. 22, figs. 5, 7; fig. C, 8) are probably reorganizing after conjugation (Campbell, 1926). These ususual forms may simulate chromidiogamy. Various phases of division are presented in figure C, 1, 5, 6, 7, and in plate 22, figures 5–7.

The *building of the lorica* is a process involving activity upon the part of the organism. Not only the production of substances which will give rise to its materials is needed, but there is also involved the moulding of this matter into a structure with a regular, characteristic species pattern. The former is a simple matter and needs only a secretory region of some sort or another, while the latter implies some sort of active constructive behavior which is periodic in occurrence and regular in application to the problem set before the organism. Both are part of the process in the Tintinnoina. The building of the lorica implies a behavior based upon structure. The concept of the mechanism of this challenging phenomenon is readily supplied by the description of a neuromotor system which furnishes the integrating and coordinating apparatus whereby the formed material is built into a characteristic lorica.

As in *Tintinnopsis* (Campbell, 1926) the animal elaborates substances which are formed into the lorica. The sources of this matrix might be, it has been suggested, (*a*) a general surface peeling (Entz, Jr., 1909), (*b*) general surface extrusion from peripheral vacuoles in the ectoplasm (Busch, 1925), (*c*) material derived from the breakup of the old lorica and reworked by the two daughters at division (Meunier, 1910), (*d*) the division of the lorica into two parts, the uppermost being taken by the anterior and the lower part by the posterior daughter (Entz, Sr., 1884), (*e*) secretion of a ring by a temporary annulus of the cytosome (Schweyer, 1909), and finally, (*f*) the lorica may be derived from a local secretion from an already

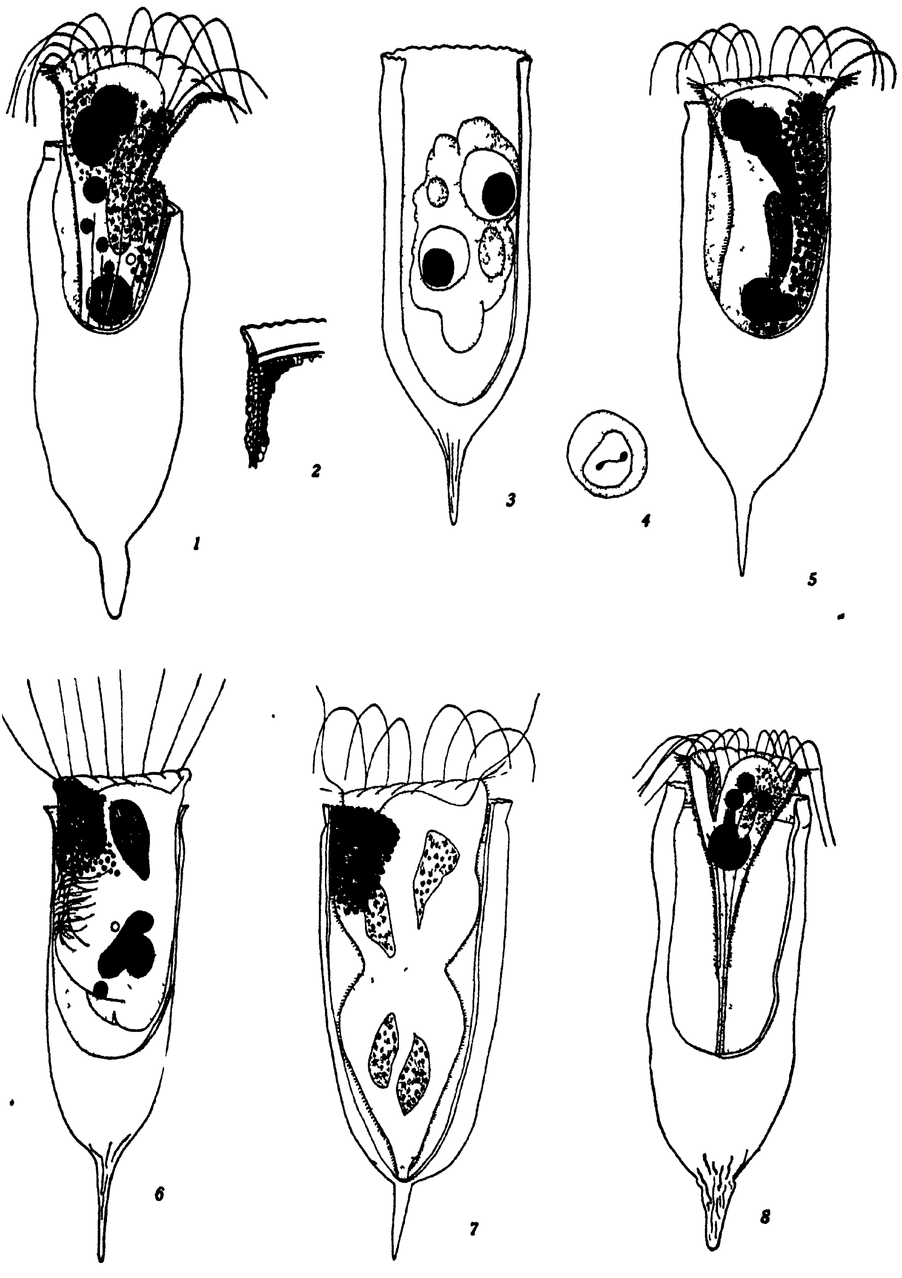

Fig. C. Camera lucida drawings of *F. serrata* showing various phases of division and other features. 1, 5, 6, 7, show division phases and especially the distribution of siderophile granules. 2, shows the structure of the wall. 3, parasitism due to *Duboscquella tintinni*. 4, isolated *Karyoclastis*, greatly enlarged. 8. Monomacronuclear form. × 260.

formed organ, as the mouth, and moulded by neuromotor organs as suggested by Kofoid (1905) and later by myself (1926). These many suggestions, some of them based upon actual observations, are difficult to reconcile.

Jörgensen (1924) regards it as only provisional that the loricas of aborally closed species are built as described by Schweyer (1909) and believes the observations of Entz, Sr. (1884) upon *Tintinnus fluviatile* to be essentially correct for these forms; he doubts that the loricas of all Tintinnoina are constructed in a uniform manner. In his account (1924) of *Rhabdonella spiralis hydria* he suggests that the loricas may be formed as short, thimble-like structures which later on, by some unknown sort of stretching or growth, acquire the form of the matured lorica with a long pedicel and elaborate apophyses. It is exceedingly difficult to imagine by what process growth might take place in those figured (1924, fig. 70*a*, *b*, *c*, *d*). It might be intussusceptional, in which case we are compelled to assume more than we can believe possible in such a non-living system as the lorica. If, on the other hand, its growth may be described by stretching the explanation of the form of the elaborate posterior pedicel of that species depends again upon some unknown and curious factor of moulding; the animal scarcely deserts its lorica and swims outside to complete its dwelling. On the whole Jörgensen leaves one much to desire regarding the formation of the lorica of *Rhabdonella*. His *Rhabdonella spiralis hydria* is probably, as he suggests is possible, only a deformed member of another species. The suppositions of Entz, Jr. (1909) and Busch (1925) that the lorica forms from surface excretions have little observation to support them. The latter author, who figures particles adhering to the surface of the cytosome of the same refractility as the lorical substance, and claims that they arose *in situ* fails, in my mind, to produce evidence that these particles were not derived from the mouth or anus. I have never seen, in an extensive series of prepared slides, any evidence of a general surface extrusion in any tintinnid. Meunier (1910) hardly accepts his own theory, advancing it only provisionally. Entz, Sr.'s (1884) account seems not to have been substantiated, although the species, *Tintinnus fluviatile,* is common.

There is definite evidence in *F. serrata,* of the source of the material used in lorica construction. The *siderophile granules* formed in dividing animals (fig. C, 1, 5, 6, 7, and pl. 22, figs. 5–7) in a dorsolateral position surrounding the mouth and often in late stages densely packed together, serve as the intracytoplasmic source of the material of the lorica. The greater mass of the material of the lorica is thus formed, another part being derived from accumulated fecal débris from the anus, especially in *Tintinnopsis.* The evidence for this is the occurrence in the wall of the lorica of skeletal remains of organisms used as food, such as coccoliths and diatoms. They would be extruded from the anus rather than the mouth. The dense mass of siderophile granules figured by Entz, Jr. (1909, pl. 16, fig. 2*c*; pl. 18, fig. 2*c*; pl. 19, figs. 5, 8*c*) and thought by him to represent chromidia may be similar granules. This mass of granules forms a dense, shield-like unit always in a constant position above the newly-forming membranelles. They appear to arise (pl. 22, fig. 5) as loose single granules in the region of constriction, which is an area of great activity, and to move into the mass one by one, or in small groups, becoming compacted later. Upon extrusion through the mouth they expand (pl. 22, fig. 6; and fig. C, 7), fuse and harden slowly, and are beaten into shape by the neuromotor organs adjacent, such as the membranelles and paroral cilia. The clear annular band (of the living animal) seen by Schweyer (1909) may be the swollen region full of this lorical-forming substance in *Tintinnus inqulinus,* and the annulus pictured (Schweyer, 1909, pl. 10, fig. 6) a diagram only of extruded lorical substance. Material is poured out of the mouth and the anus as may be observed in the living organism. Kofoid (1905) was struck with the possible relation existing between a localized secreting area and the general prevalence of annulations and coiling in the loricas of *Coxliella* (*Cyttarocylis*) *pulchra,* which he described from the San Diego plankton. This material is within the reach of neuromotor organs and may be transferred by their activity to form the rugose refractile layered alveolar structure of the lorica, which is a colloidal aggregation and formed from materials derived from the animal. This ebullition of foamy material especially from the mouth provides an asymmetrically placed source, and, as Kofoid observes (1905), the animals in common with other ciliates rotate about their long axes and thereby may distribute this matter symmetrically by their membranelles and cilia. The material may then be transferred by the neuromotor organs which by their activities shape up the lorica. The lorica reflects, in its permanent structure, the manner of its formation, as in *Coxliella* and *Climatocylis* where strong coiling is evident, or in *Favella,* where rugose pleats are prominent and denticles are provided about the oral lip. *Neuromotor* *organs are the moulding agents.* Spines, apophyses, and other elaborations are the work of these organs, at binary fission. The serrations and repeated annulations of some, as the denticles of *Favella serrata,* are probably the work of the inhabitant itself as noted by Kofoid (1905) in *Tintinnus serratus* and *Tintinnopsis reflexa.* We suggest that the strong correlation between the number of denticles about the oral margin of *F. serrata* and the number of ciliary lines, especially of the longer paroral cilia, is of the greatest importance in this connection. The pedicel may be the work of the posterior daughter at division, and its knobbed, spiked, or irregular end the finishing stroke of the tools below. These correlations are too significant to be passed unnoted. As I have suggested in another connection the regions of the greatest differentiation of the lorica in all the Tintinnoina are those which are in contact at the time of the formation of the lorica, or at all times, with the neuromotor organs.

The regularity of pattern resulting from the behavior of the neuromotor organs is exemplified in the regularity of the loricas in the many finely separated species of tintinnids. The process of speciation in this large group has gone very far as even a cursory examination of Brandt's monograph (1906, 1907) demonstrates. The determination of this speciation in Brandt's monograph is founded upon the structural modifications of a single organ of the animal, the lorica. But the lorica reflects in its construction the fundamental, peculiar organization of the animal within it (Campbell, 1926) and it shows the specificity, not only of the structures used in its formation but equally of the behavior which formed it. It may therefore be held that behavior-pattern which is uniform in action within the species, and periodic in its expression in a given form, may aid the understanding of the manner of formation of the lorica not only in *Favella serrata* and *Tintinnopsis,* but also in the Tintinnoina as a whole. It finds its anatomical basis in a central, dominating neuromotor apparatus, within the complex body of these multicellular protozoans, and in the number, position, and relations of the cilia and membranelles, the tools available for moulding the lorica in the period of formation.

The stereotyped behavior of the neuromotor organs is repeated at each bipartition. Possibly the widely prevalent asexual reproduction and the repetition of countless generations by this method, each with a similar genetic, chromosome inheritance, may favor the regularity of behavior which accounts for the little dissimilar and narrowly speciated loricas of each genus in every sea. Sexual behavior by conjugation or by phases of internal reorganization may then account for the mechanism of the evolution of the lorica and its inhabitant, provided a modern conception of heredity applies in the protozoa (Kofoid, 1923), as well as in the Metazoa; this unites in one system all such organisms.

SUMMARY

Favella is a largely neritic genus of the Tintinnoina. Its cytosome is trumpet-like with a flattened, spiral frontal field. There are two macronuclei and two micronuclei. There is a complex neuromotor apparatus of intracytoplasmic fibers linking the motile organs with a central neuromotorium.

The large loricas by their form and structure are adapted to pelagic life. The high specific surface and lightened structure add to the buoyancy of the organism. These loricas are formed from material elaborated in the cytoplasm and discharged largely by the mouth, and distributed and fashioned by the neuromotor organs into the characteristic lorica which is of high survival value. The loricas afford an excellent basis for the systematic allocation of the species in the Tintinnoina.

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EXPLANATION OF PLATES

All figures are drawn with a camera lucida from preparations fixed in hot Schaudinn and stained in iron-haematoxylin, except figs. 5–7, which were stained in borax-carmine.

PLATE 21

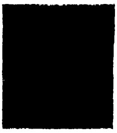
× 422

Fig. 1. Lorica of *F. serrata,* cut away to show lumen.

Fig. 2. Cytosome of *F. serrata,* showing general features.

Fig. 3. Isolated macronucleus and micronucleus early in division.

Fig. 4. A small portion of the wall to show primary and secondary alveoles.



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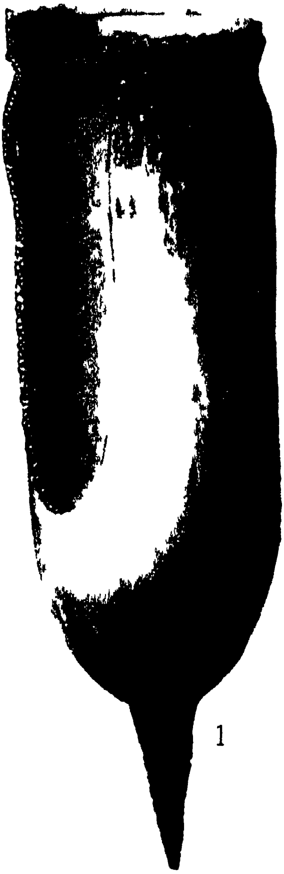

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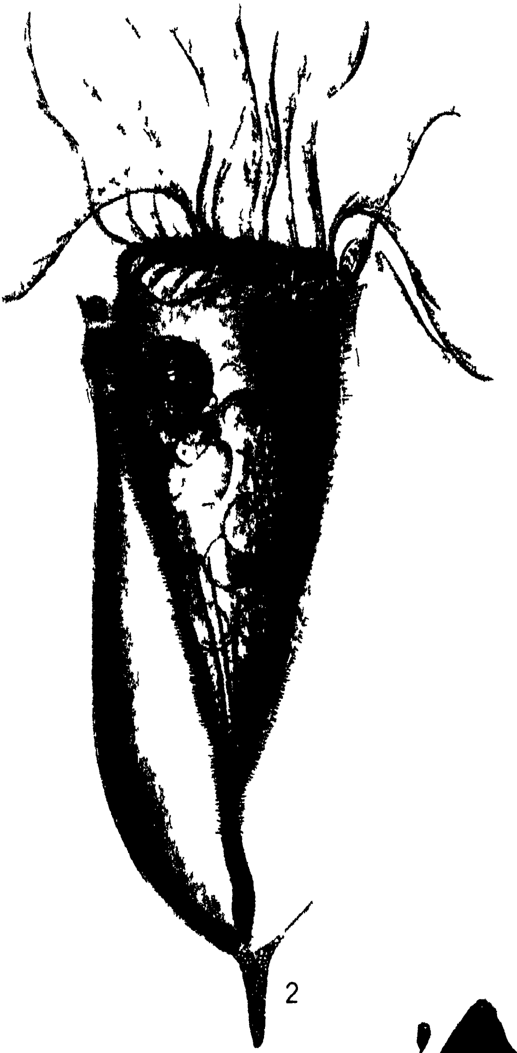

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3

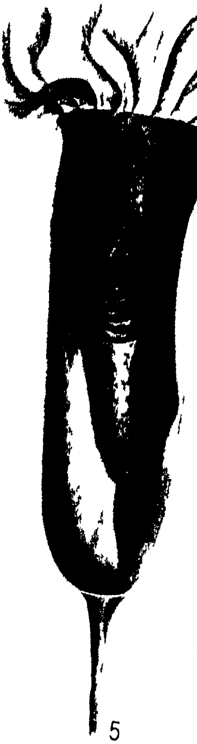
PLATE 22

× 338

Fig. 5. Dividing individual showing the accumulation of siderophile granules about the cytostome.

6. The granules have become massed about the cytostome and compacted closely. The new mouth is well advanced.

Fig. 7. Same individual as fig. 5, but rotated 90°, showing the extent of the siderophile granules and their compacting about the mouth within the cytosome.



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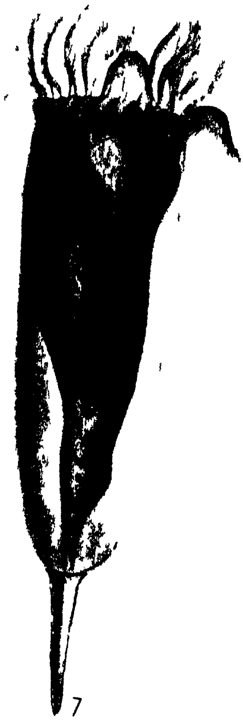

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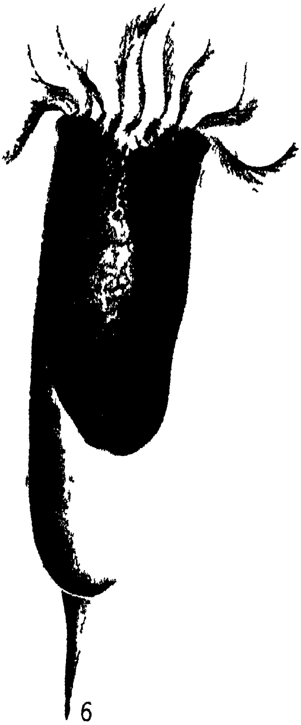

6

THE ELECTRICAL CONDUCTIVITY OF PROTOPLASM AND A NEW METHOD OF ITS DETERMINATION

BY

SAMUEL GELFAN

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THE ELECTRICAL CONDUCTIVITY OF PROTOPLASM AND A NEW METHOD OF ITS DETERMINATION

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SAMUEL GELFAN

Electrical conductivity of protoplasm is not only in itself an important property to be ascertained but it may also be used as a means of studying other biophysical properties and phenomena. In physical chemistry, for example, it is used as a means of studying such phenomena as the degree of ionization and rates of reaction. An accurate determination of the electrical conductivity of protoplasm and the successful utilization of this property in the study of biological problems depends upon the technical perfection of the methods employed. As we shall see farther on, some of the announced results of its use have proved unreliable because of unsatisfactory methods. It is the purpose of this paper to describe the method employed by the writer to determine the electrical conductivity of protoplasm and to give the results thus far obtained by this method.

Though electrical conductivity of tissues has been measured by several investigators (Galeotti, 1903; Kodis, 1901), the penetration of the cell wall by the electrodes, and the conductivity of the protoplasm of any single living cell independent of the cell wall have never been directly determined. In the methods hitherto most frequently employed for measuring the conductivity of protoplasm, the electrodes have been placed either in a solution containing living cells, or close to the membrane of a cell (Osterhout's method). In either case the assumptions are made that the cell wall has a very low resistance and that it is freely permeable to ions. Such assumptions are necessary or the measurements would not mean anything. But to assume that the cell wall, especially that of plants, has a lower resistance than the medium in which it lives is unwarrantable, and most of the evidence indicates that the cell wall is not normally permeable to ions.

At best Osterhout's (1918) method of measuring conductivity can only indicate conductivity changes due (according to Osterhout) to changes in permeability to ions which are brought about by injuring the cell in various ways. This method has been criticized, and Osterhout (1925) himself now holds the view that protoplasm is not permeable to ions, and that only the undissociated molecules can enter the protoplasm. He explains his results on conductivity changes in relation to changes of permeability to ions as due to the abnormal conditions induced in the cell by the electric current, the normal cell not being permeable to ions (Osterhout, 1925).

By the method that is described here, it is possible to insert into the interior of a single living cell (without greatly injuring the cell) electrodes that are minute enough to enter the cell and at the same time so to function that direct and accurate electrical measurements can be made of the electrical conditions attending the stimulation and the normal functioning of that cell. Mounted on a micro-manipulator, non-polarizable micro-electrodes are used with a very sensitive galvonometer for measuring the resistance.

The writer (Gelfan, 1926) began to perfect a non-polarizable micro-electrode on the basis of the work already done by Taylor (1925). About that time Ettisch and Peterfi (1925) described a non-polarizable micro-electrode. The one described here is considerably different. The former is really a micro-calomel cell, whereas the latter is a Ag, AgCl, KCl system. The methods of construction are not only independent, but considerably different.

THE ELECTRODE

The non-polarizable micro-electrode consists essentially of three parts, the *micro-pipette* (fig. 1*b*), the tip of which penetrates into the cell or microörganism, the *main pipette* (fig. 1*a*) into the shank of which the micro-pipette is sealed, and the AgCl electrode (fig. 1*e*). The latter is sealed into a glass plug (fig. 1*c*) which is ground to fit perfectly the other end of the main pipette. The main pipette and the capillary micro-pipette are both filled with the same agar which is impregnated with KCl. The system then is Ag (fig. 1*d*), AgCl (fig. 1*e*), KCl in agar (fig. 1*f*).

The agar, as will be seen from the account of its preparation, is not a gel at room temperatures but a milky, very fine white precipitate. The coil of the AgCl (fig. 1*e*) is not dipping into a gel, then, but into a fluid (fig. 1*f*). If the agar in the system were a gel, only that much of the main pipette would be filled with agar as would be necessary to completely cover the micro-pipette that is protruding from the pipette shank. The rest of the main pipette, into which the AgCl coil is dipping, would contain a KCl solution of the same concentration as the KCl in the agar.

The main pipette (fig. 1*a*) is drawn out from glass tubing to a suitable size for the the pipette holder of the micro-manipulator. The accompanying figure (fig. 1) shows practically the natural size of the entire micro-electrode, except that, obviously, the terminal is relatively enlarged.

For the micro-pipettes, capillary pipettes are drawn out from clear quartz tubing to about 0.5 mm. in diameter. These in turn are drawn out at one end to minute points over an oxygen microflame. The tips of these pipettes, which have now openings of about 1–2μ in diameter, are bent almost 90° as is indicated in the figure. Filling these small pipettes with agar is the most difficult task. That end of the micro-pipette which is not drawn out to a fine point is inserted into the nozzle of a 10-cc. Luer syringe and is sealed to the latter with de Khotinsky cement. It has been found advantageous to insert the pipette far enough so that it just makes its appearance in the barrel of the syringe. The syringe, which is fastened by a clamp to a ring stand, is now filled with the warm agar. As pressure is applied on the plunger, the agar can be seen slowly advancing in the pipette toward the fine tip, and as soon as it reaches the end, with no release of the pressure, the micro-pipette is broken off. After examining the pipette under the microscope and ascertaining that there are no bubbles anywhere, it is sealed into the main pipette shank with dental cement.

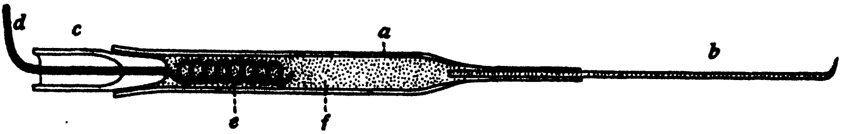

Fig. 1. The non-polarizable micro-electrode. × ⅘: *a*, main pipette; *b*, micro-pipette; *c*, plug; *d*, silver wire; *e*, silver wire coated with silver chloride; *f*, agar impregnated with KCl.

THE AgCl ELECTRODE

Into the glass tubing, which is to constitute the 'plug' (fig. 1*c*) of the main pipette, a specially prepared c.p. silver wire of about 1 mm. in diameter is sealed. The plug is then ground to fit the main pipette. After the wire is thoroughly cleaned and washed, it is placed in a 0.75 N c.p. HCl solution, and, making it the anode, a 4-milliampere current is run through the solution for five hours. The silver wire is by this process smoothly and evenly coated with AgCl. Inasmuch as it is desirable to have both electrodes of any pair that is to be used as nearly alike as is possible, both 'plugs' containing the Ag wire are suspended in the HCl solution and are both made the anodes against another silver wire as a cathode. After the deposition of the AgCl, the electrodes are washed several times in distilled water and are then left for two days in conductivity water to remove any traces of HCl.

It may be suggested here, if any difficulty is encountered in sealing the silver wire into the glass plug, that a platinum wire be used instead, since it has a higher melting point. Silver may be deposited electrolytically in a K Ag CN solution. AgCl may then be deposited by the method outlined above.

PREPARATION OF AGAR

The dry agar is cut up into small chips and placed in a muslin bag. This muslin bag containing the chips is suspended in a large funnel of running distilled water. The agar is first allowed to swell for twelve or more hours. Platinum electrodes are then placed one inside the bag and one outside but close to the bag. Then 110 volts of direct current are sent through for about five hours, the direction of the current being then reversed and allowed to pass once more for an equal length of time, thus removing both anion and kation impurities. This is a very effective and yet simple method of dialysis. A one or two per cent solution of agar is prepared, and after having been filtered it is made $\frac{N}{10}$ with KCl.

After heating the agar thus prepared once or twice in order to solate it, it was found that upon cooling it did not return to the gel state as would normally be expected. Instead it formed, upon cooling, a very fine precipitate having the appearance of milk, which on standing slowly settled with the appearance of water above it. Obviously the agar lost water and this may have been due to the complete removal during the dialysis of some ion that is necessary for the gelation of the agar. (It must be remembered, however, that before the pipettes are injected the agar is heated to a sol.) So far as the writer is aware, an agar that is not a gel at room temperature, has not been reported before.

After the micro-pipette has been injected with the agar and sealed into the main pipette shank with dental cement, the main pipette is immediately filled with the same agar, and without causing any bubbles the plug containing the AgCl coil is inserted into the main pipette and likewise sealed with dental cement. The entire system is now air-tight. The assembling of the electrode is made in the absence of sunlight to avoid the decomposition of the AgCl. The main pipette is painted with India ink so that experiments may be carried out in sunlight without the danger of decomposing the AgCl. The electrodes are now mounted on a Taylor (1925) micromanipulator.

Since the points of the electrodes are so minute, the resistance of the electrodes becomes very great. Consequently the micro-oscillator and telephone method of measuring resistance cannot be used because of lack of sensitivity. Instead, the following method is employed. The electrodes are connected in series with two or three dry cells, a two-way switch, and a Leeds and Northrup galvanometer whose sensitivity is 29,200 megohms. Because of the extreme sensitivity of the galvanometer, the wires of the system must be well insulated. The galvanometer is then standardized with known concentrations of KCl, from 0.1 N to tap water. This is done by immersing the points of the electrode in a drop of the solution that is hanging from a coverslip over the moist chamber (fig. 2).

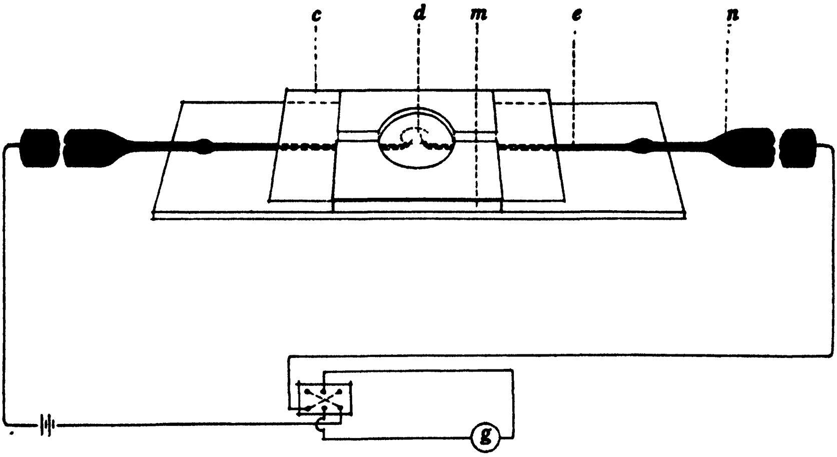

Fig. 2. Moist chamber which rests on stage of microscope, micro-electrodes, and electrical connections: *c*, coverslip over the moist chamber; *d*, drop of liquid or organism hanging from coverslip in the moist chamber, and into which the points of the electrodes are dipping; *m*, moist chamber; *e*, micro-electrode; *n*, part of the electrode which is held in position on the micromanipulator by pipette holder.

The electrodes are always placed the same distance apart (measured by ocular micrometer) in the living cell as well as in the salt solution in any one set of experiments. After having observed the amount of deflection on the galvanometer for each concentration, a curve may be plotted with concentration of KCl as ordinates and deflection of the galvanometer as abscissa. The salt concentration in the living organism is determined by finding the corresponding concentration on the above curve, from the amount of deflection as observed in the case of the organism. That is, the deflection, observed in the case of the organism, when found on the curve made from the standardization of the galvanometer with KCl solutions, gives the concentration of KCl that corresponds to that deflection. The specific conductivity of the organism would then be equal to the corresponding concentration of the KCl.

CONDUCTIVITY OF PROTOPLASM

By the method outlined above, the electrical conductivities of the large plant cell of *Nitella,* and of the ciliate Protozoa *Paramecium, Oxytricha, Blepharisma,* and *Stentor* were measured. It is obvious that the problem of the cell wall or plasma membrane is here eliminated since the electrodes are inside the living cell. The measurements consequently indicate the extent to which the current can pass through the fluid protoplasm. The following table lists the forms, the concentration of KCl to which their internal conductivity corresponds, and the specific conductance in reciprocal ohms at 18° C., obtained by me under the experimental conditions as indicated above.

	N	KCl
Nitella	.07	.0077
Paramecium	.06	.0068
Oxytricha	.05	.0058
Blepharisma	.04	.0047
Stentor	.035	.0041

The figures given in the table are the averages of many measurements, but in any one single measurement the deviation normally is never greater than 20 per cent. During the measurement there is usually a slight fluctuation back and forth of the deflection of the galvanometer, due presumably to continual metabolic changes in the organism. Unless the measurements during any one experiment were consistent, they were discarded. It may be mentioned here that, as the electrode is inserted into the organism, any minute amount of water that might be brought in as a result of the diffusion of water from the medium around the outside wall of the micro-pipette would not influence the results, since the conductivity of the protoplasm is higher than that of the medium.

In contrast to the Protozoa, the plant cell of *Nitella,* because of its tough wall, offers considerable resistance to the penetration of the electrodes. Likewise, it is difficult to remove the electrodes from the interior once they are in. This seems to be brought about by the fact that a very small amount of protoplasm congeals around the point of entrance of the electrode, so that the wound is immediately healed and the cell is left intact. The streaming of protoplasm in *Nitella* is an excellent criterion of the condition of the cell. In young and healthy cells the movement is rapid and vigorous. Immediately upon penetration of an electrode into the cell, the streaming of protoplasm ceases, and, as we have observed with dark field illumination in *Nitella,* the Brownian movement likewise stops for a very brief moment. The streaming, although it shortly commences again, does not attain its normal rate until about two minutes after the stopping. The small current that passes through the cell does not seem to have any effect on the streaming. But it has been observed in previous (unpublished) experiments with larger platinum electrodes and a stronger current, that upon closing the circuit the streaming immediately stops, but after breaking contact the streaming starts again and after two minutes has attained its normal rate.

The protoplasmic layer in the cell of *Nitella,* which is very thin, lies close to the cell wall. The rest of the interior of the cell is taken up by the large vacuole containing the cell sap. Because of the extreme thinness of the layer of protoplasm, the electrodes are no doubt dipping mostly into this vacuolar sap. It is consequently the conductivity of the cell sap that is measured in this case. But the concentration of electrolytes in the protoplasm of *Nitella* is probably the same as in the cell sap.

The determinations of conductivity for *Nitella* obtained by me are somewhat lower than those obtained by Hoagland and Davis (1923) for this plant. In their experiments, the cells are broken and enough sap collected for a measurement. The conductivity of the cell sap according to their measurements corresponds to about a decinormal solution of KCl whereas the writer's measurements by the above outlined method give a conductivity for the interior of the *Nitella* cell that is equivalent to a 0.07 N KCl. For their experiments, Hoagland and Davis used large cells varying in length from one-half to three inches, whereas the cells used by the writer are relatively much smaller (never more than 0.25 of an inch in length), and, as they state in one of their recent papers (1926), it is possible that the small cells contain sap of lower concentration than that of the sap in the large cells.

It must be remembered that in the writer's experiments the electrodes are in the interior of the living cell. It is also possible that the introduction of the electrodes produces some injury and, as Hoagland and Davis (1923) have shown, following the slightest injury there is a loss of ions from *Nitella* cells into the medium.

In measuring the conductivity of the protozoan cells, they are first isolated from the culture by means of a small pipette and placed on the coverslip that is to go over the moist chamber with a small drop of the medium solution (aquarium water). Enough water is then drawn off from this small drop to reduce the activity of the animal to such an extent as will make its 'capture' by the micro-manipulation of the electrodes not difficult. There is little difficulty encountered in inserting and withdrawing the electrodes from the cytoplasm of the Protozoa that are listed above. The small current that passes through their protoplasm seems to have no injurious effect. Even after many manipulations, the organisms will survive as long as care is taken that the water surrounding them does not dry up.

Although the electrode system is non-polarizable with respect to a KCl solution, it is possible that a slight amount of polarization may take place when the electrodes are in the organism, since we do not definitely know all the ions that carry the current in the living cell. If polarization does take place, however, it must be very small for the following reasons. Protoplasmic streaming, which is very marked in *Nitella,* should have the same effect in eliminating polarization as the stirring of a solution in which the conductivity is measured by direct current. Another important factor is the minuteness of the electrode points (1–2μ), and also the small distance separating the electrode points during a measurement (usually about 125μ). If polarization should take place, however, it would mean, consequently, that the conductivities might be slightly higher than our measurements indicate.

ELECTROLYTIC CONDITIONS OF CELL WITH RESPECT TO THE MEDIUM

It will be seen from the above table of conductivities of living cells that the concentration of electrolytes in the interior of the cells is considerably greater than in the medium, since they are fresh-water forms. This is in agreement with the already mentioned work of Hoagland and Davis. Thornton (1912) also found that the conductivity of living bacteria is greater than that of the medium. The concentration of electrolytes in the interior of the bacterial cell must consequently be greater than in the medium. His method of measuring electrical conductivity of bacteria is based on the principle that bacteria will orient themselves in an electric field only in a solution having a lower conductivity than the bacteria. It follows, then, from the above considerations, *that the normal condition of the fresh-water organisms is to continually live in a solution which is hypotonic with respect to that cell.*

The concentration of salts found in the above organisms is also somewhat indicative with regard to the colloidal structure of protoplasm. As Lepeschkin (1924) has pointed out in the case of plasmodia which contain 0.3 per cent water soluble salts, protoplasm must be a hydrophil-colloid. Such a salt concentration (0.3 per cent) would be sufficient to coagulate a hydrophobe-colloidal solution.

EFFECT OF INJURY

It was found by the writer that injuring the cells decreased their conductivity. The cells were not injured by chemicals but mechanically, by pulling and stretching them with the electrodes. By noting the deflection of the galvanometer, it could be observed that the *conductivity continued to decrease with increase of injury.* Upon severe injury, the conductivity of the cell would approach to nearly that of the medium, which is practically the same as that of tap water. The decrease in conductivity upon injury was observed in all the animal cells listed above. The plant cell *Nitella* cannot be injured in this way without causing it to collapse.

These results are the opposite to those obtained by Osterhout (1922). In his experiments with the marine alga *Laminaria,* he finds that the conductivity increases with injury, the increase in conductivity indicating an increase in permeability. How are we to explain these seemingly conflicting results?

Conductivity of protoplasm is a measure of the ability of the electric current to flow through the protoplasm. But it must be remembered that in the method used by Osterhout, the current, if it does appreciably go through the cell, must pass from the sea water, which is a good conductor, through the wall and interior of the cell. Such a measurement could indicate the conductivity of protoplasm only when the cell wall is entirely permeable to ions and offers no resistance to the current. But that is not the case, Osterhout (1925) himself now holding the view that protoplasm is not permeable to ions. His results, consequently, mean that upon increase of permeability caused by injury, the current can pass *through the cell wall* with greater ease, but it does not indicate that the conductivity of protoplasm has increased.

As has been shown above, the fresh-water protozoans and the plant cell *Nitella* live in a medium whose concentration of electrolytes is lower than that of the cells. It has also been pointed out that upon injury there is a loss of electrolytes. When these fresh-water protozoans are therefore injured, there is a temporary loss of their ability to hold electrolytes at a greater concentration than that of the medium, and because of this loss of electrolytes into the medium, the conductivity of protoplasm would consequently decrease. The ionic concentration of the medium has increased but in the interior of the cell it has decreased.

Upon this basis the conflicting results of other investigators may be explained. Shearer (1919), for example, found that upon injury the conductivity of a heavy bacterial suspension increased. As Thornton's (1912) results indicate that the bacteria contain a greater concentration of electrolytes than the medium, it was probably the conductivity of the medium, in which the bacteria were suspended (in Shearer's experiment), that increased, whereas the conductivity of the interior of the bacterial cells actually decreased. This, of course, assumes, as Thornton's experiments indicate, that the electrolytic content of the bacterial cells is actually greater than that of the medium.

The high resistance offered by living cells to the electric current must, therefore, be due mostly to the impermeability of the cell wall to ions. But the protoplasm of the interior of the cell, as has been shown, conducts the current to a very appreciable extent.

Roth (1897), Bugarszky and Tangl (1897), and Stewart (1897) concluded that living corpuscles hardly conduct the current at all.

But Höber (1910), using a method wherein there was no question of ions being able to leave the cell, found that the internal conductivity of blood corpuscles was equivalent to about that of a 0.1 N KCl solution. The first method he used is based on the fact that the capacity of a condenser is increased when a conducting stratum is introduced into the dielectric between the condenser plates. By the second method (1912), which is based on the fact that a conducting body, placed in the center of a coil of wire through which a rapidly alternating current is passed, diminishes the strength of this current by damping the vibrations, Höber found the internal conductivity of blood corpuscles to be equivalent to that of a 0.1 to 0.4 per cent NaCl solution.

It will be noted that there appears to be a correlation between the specific conductivity and the degree of relative activity of the organisms used in these experiments. This was suggested to the writer by Dr. Kofoid. *Paramecium,* which has the highest conductivity, is the most active of the protozoans. *Oxytricha* is less active, *Blepharisma* is a sluggish animal keeping near the bottom of the aquarium most of the time, and *Stentor* is sessile. *Nitella,* although a plant cell and therefore not active at all in the sense of moving about, nevertheless represents a high degree of activity in another respect. Protoplasmic streaming, which is a characteristic of many living cells, is very marked in *Nitella.* It is much more rapid and vigorous in *Nitella* than in the protozoans tested by the writer, and in this respect *Nitella* may be said to be more active than *Paramecium,* and consequently to have the higher conductivity.

It is possible, then, that the concentration of dissociated molecules in the protoplasm is in some way related to the activity of the organism. Before such a relation can definitely be established, of course, the conductivities of many more organisms will have to be known.

SUMMARY

1. A method is described whereby the electrical conductivity of protoplasm can directly be measured by penetration into the interior of the cell by non-polarizable micro-electrodes.

2. The protoplasm of those forms that were measured conduct the current to a very appreciable extent.

(The conductivities are expressed in reciprocal ohms at 18° C.)

Nitella (cell sap)	0.0077
Paramecium	.0068
Oxytricha	.0058
Blepharisma	.0047
Stentor	.0041

3. The electrolytic content of these cells is greater than that of the medium.

4. The internal conductivity decreases with injury.

5. It is possible that the concentration of dissociated molecules in the protoplasm is in some way related to the activity of the organism.

The writer wishes to express his gratitude for the advice and criticism received from the following members of the University of California: Professors G. E. K. Branch and A. R. Olson, of the Department of Chemistry, Professor D. R. Hoagland, of the Department of Plant Nutrition, Professor Leonard Loeb, of the Department of Physics, Dr. D. Greenberg, of the Department of Biochemistry, Professor C. A. Kofoid, of the Department of Zoology; and to Professor C. V. Taylor, of Stanford University.

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KOFOIDIA, A NEW FLAGELLATE, FROM A CALIFORNIA TERMITE

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S. F. LIGHT

This paper contains an account of a remarkable multiflagellate mastigophoran, *Kofoidia loriculata* gen. nov., sp. nov., from the intestine of the termite, *Kalotermes simplicicornis* Banks. Living in continual association with *Hoplonympha natator* Light (1926*a*), *Kofoidia* differs from it in all save the fundamental flagellate characters and in the possession of many flagella, which would place them both in the order Hypermastigida as heretofore constituted. In contrast to the elongated, rigid body of *Hoplonympha* we find in *Kofoidia* a spherical body, massive, yet flexible (fig. A; fig. E). The cytosome is very large with maximum of volume to minimum of surface, and, while it possesses a definite periplast and maintains, ordinarily, a fairly constant shape, yet it is sufficiently metabolic to ingest great quantities of wood in masses even much greater in length than its ordinary diameter (fig. E, 6, 7, 9). In its locomotor organs we find nothing of the bilaterality characteristic of *Hoplonympha* but a peculiar spiral grouping of flagellar bundles and other neuromotor structures. These remarkable, permanently integrated, cylindrical bundles of flagella (fig. A) are termed here *loricula* (sing. *loriculum,* diminutive from *lora,* a whip of many thongs). They suggest in many ways the cirri of the ciliates and represent by far the most highly developed motor organs yet reported among the flagellates. But with all their structural development they function very slightly for actual progression and, from the point of view of effective locomotion, are negligible as compared with the motor organelles of *Hoplonympha, Staurojoenina* (Kirby, 1926), and many others of the parasites of the termites. Hampered by the comparatively great weight and size of the cytosome, *Kofoidia,* though thus admirably equipped for locomotion, remains more or less stationary or at most moves but slowly from place to place.

Binary fission in the two species, *Hoplonympha natator* and *Kofoidia loriculata,* presents equally striking points of difference. *Hoplonympha* agrees with the higher hypermastigotes, as at present known, in that its nucleus moves forward to the vicinity of the blepharoplast for the mitotic process and the neuromotor structures are divided between the daughter organisms. *Kofoidia,* on the contrary, imitates *Lophomonas* in that, at the time of division, the nucleus deserts the old neuromotor system, or at least the motor organelles, and undergoes division at the opposite end of the body (pl. 24, fig. 13). The two new neuromotor systems arise by outgrowth from the ends of the paradesmose (pl. 24, figs. 12, 13) and the old motor structures, while temporarily functional, gradually disintegrate (fig. H, 2, 3) and are resorbed.

Aside from the similarity in their behavior at time of fission, structural characters such as the form of the body, the general position of the flagella, etc., point to *Lophomonas,* an inhabitant of the intestine of cockroaches, far removed as it is, as the nearest known relative of this new multiflagellate type.

The profound contrast in form, structure, habit, and reproduction existing between the two organisms, *Kofoidia* and *Hoplonympha,* which we have every reason to believe have been in the closest of associations for millions of years, would seem to depend therefore, in part at least, upon differences in the stock from which each has developed and not to represent divergent response to a common environment. This would seem to indicate a remarkable genetic conservatism which makes all the more striking the diversity of form which we find among the Protozoa of the termites and suggests the probability that the original infection of the ancestral termite hosts must have been from an already differentiated flagellate fauna. This would mean that the elements of the present-day faunas of the termites represent several more or less distinct phylogenetic stocks. Such a conclusion would serve to lighten the difficulties of understanding why such a diverse fauna should develop in so uniform an environment.

If we consider the great differences between *Lophomonas* and *Kofoidia* in the light of the tremendous period of time which has elapsed since the termites and the cockroaches separated from some common problattoid stock, the marvel is not that there are such differences but that there remain sufficient resemblances to enable us to recognize the relationship. As it is, the differences are so great as to make necessary the erection of a new family to receive the new genus *Kofoidia.*

Kofoidia is one of the largest of the Protozoa of the termites and is by far the largest inhabitant of the intestine of *Kalotermes simpli-*

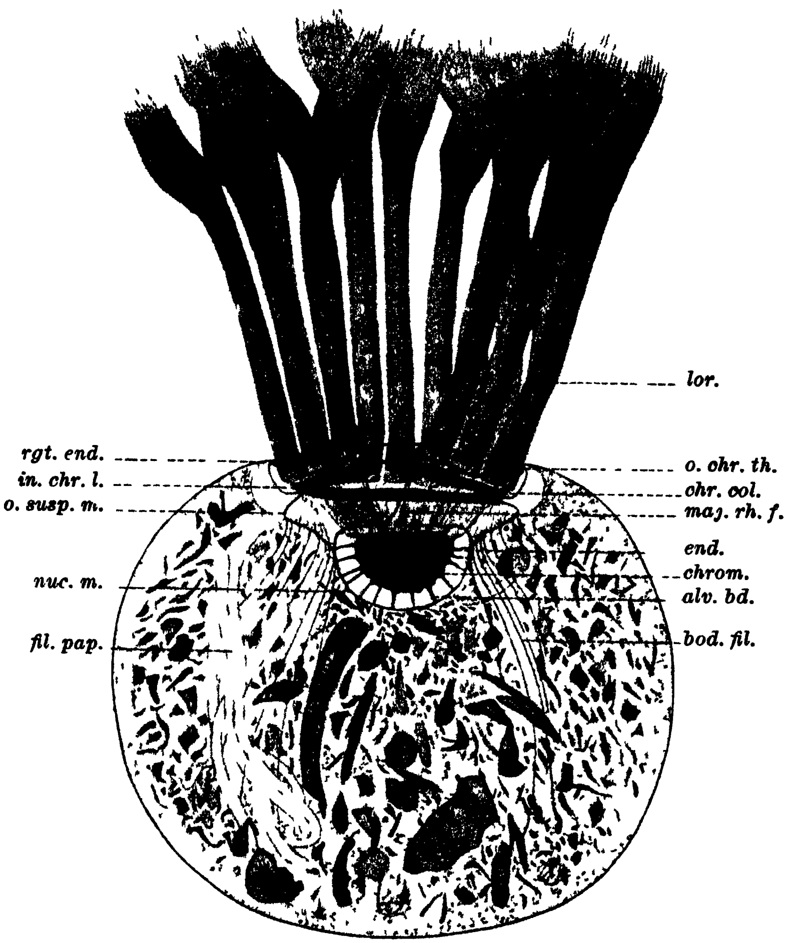

Fig. A. A somewhat diagrammatic figure of *Kofoidia loriculata* gen nov., sp. nov., from *Kalotermes simplicicornis* Banks. × 1500. The loricula are shown at maximum size and the cytosome below the average size. Abbreviations: *alv. bd.*, chromatic boundary of an alveolus of the clear zone of the nucleus; *bod. fil.*, body filaments which run from the outer suspensory membrane to all parts of the cytosome; *chr. col.*, chromatic collar; *chrom.*, the chromatic mass of the nucleus; *fil. pap.*, ingested filter paper fiber; *in. chr. l.*, internal chromatic line within the collar; *end.*, endosome in clear halo; *maj. rh. f.*, major rhizoplast fiber of left end which is also the terminal of the inner suspensory membrane and parallels the right terminal rhizoplast to the nuclear membrane; *nuc. m.*, nuclear membrane; *o. chr. thr.*, outer chromatic thread which runs around the outer surfaces of the bases of the loricula and, at either end, to the nuclear membrane as right and left terminal rhizoplasts respectively; *o. susp. m.*, outer suspensory membrane, shown here in optical section; *rgt. end.*, right, i.e., outer end of the collar.

Note the clear space above between the outer suspensory membrane and the cytoplasm, the attachment of the membrane to the collar and its relation to the chromatic thread. See fig. D for the complete course.

Note the deep incurvature of the left end of this membrane (right, as seen here) of the collar, chromatic thread, and loricular series, also numerous, slender, eosinophile, minor rhizoplast fibers running through the inner suspensory membrane to the nucleus with a few siderophile major rhizoplast fibers interspersed. Note the light basal plates of the loricula and the line marking the outer limits of the hypostome-like mound of cytoplasm commonly found within the loricular area in fixed specimens.

cicornis where, in mass at least, it seems to be dominant. Its great size, its typically opaque appearance due to the great number of wood fragments it ordinarily contains (fig. A), as well as the striking movements of the loricula (bundles of flagella, *lor.*, fig. A), at once attract the eye even in this museum of protozoan marvels.

The same materials and methods were used here as in the study of *Hoplonympha natator* (Light, 1926*a*) save that a *short* stain by the iron haematoxylin method following fixation for about twenty to thirty minutes in Bouin's at 56–60° C. was found to give excellent contrasts, particularly when followed by a counterstain with eosin or light green. Osmic fixation presents a considerably different but consistent picture as regards the neuromotor system, which will be considered later.

This remarkable genus has been named for Dr. C. A. Kofoid, whose outstanding work in this and other fields of protozoology needs no recounting here.

Kofoidia loriculata gen. nov., sp. nov.

Plates 23, 24, figures 1–16; figures A–H

Shape and size.—*Kofoidia* is characterized by its massive, approximately spherical form. It is 120 (60–140) microns in diameter. Specimens distorted by elongated particles of wood may reach a length of 200 microns (fig. E, 9). In artificial media individuals are rarely seen to take on an elongated ovoidal form. This seems to be the case at times with numbers of individuals in the intestines of termites existing under abnormal conditions. Here the neuromotor system is always at one end, usually the smaller one (fig. E, 5). In the case of individuals elongated by large wood fragments the neuromotor system lies in varying relation to the temporary long axis (fig. E, 6, 7, 9) which would seem to indicate a persistent polarity marked by the location of the neuromotor system.

When placed in artificial media the particles of wood are often thrown out in some manner not understood, the result being a great decrease in size and a cytosome of clear cytoplasm. Some individuals also throw off large portions of the cytoplasm as in *Trichonympha* (Kofoid and Swezy, 1919) resulting in an abnormal disproportion in size between cytosome and neuromotor system. Individuals from the intestines of starved termites show correspondingly great decrease in the size of the cytosome (cf. figs. 3 and 5, pl. 23).

Orientation.—The series of flagellar bundles described below may be employed for purposes of orientation. The end of the organism bearing the neuromotor system will be termed the anterior; the side on which the spiral is open, the ventral (fig. A; pl. 23, figs. 4, 5). This will make that side the right on which the loricular line ends farthest from the center of the spiral and the left that on which the end is within and ends in a curved tip (pl. 23, fig. 4; pl. 24, figs. 6, 15).

Neuromotor system.—The neuromotor system of *Kofoidia* is so unique as to require the use of new terms or unusual uses of old ones. Certain structures probably having a supporting or anchoring function (cytoendoskeleton of Grassi, 1917; Kirby, 1926) have been included with the more definitely neuromotor structures because of their close integration with them.

The neuromotor system is located near one end which has been designated on that basis the anterior end. It consists of (1) 8–16 cylindrical flagellar bundles, termed *loricula* (*lor.*, fig. A), arranged in a curved linear series, usually making from one to two turns of a leiotropic spiral (fig. E, 6, 9); (2) a broad, more or less chromatic band, the *chromatic collar* (*chr. col.*, fig. A) underlying the course of the loricula; (3) the strongly siderophile *outer chromatic thread* (*o. chr. thr.*) closely applied to the outer surfaces of the intracytoplasmic bases of the loricula; (4) the *left* and *right terminal rhizoplasts* (fig. A) continuations of this thread to the nucleus at either end; (5) a *suspensorium* made up of an *inner* and an *outer suspensory membrane* (*in. susp. m.*, *o. susp. m.*) connecting the neuromotor spiral throughout its length to the nucleus; (6) a series of slender, eosinophile fibers, the *body filaments* (*bod. fil.*), arising from the outer suspensory membrane

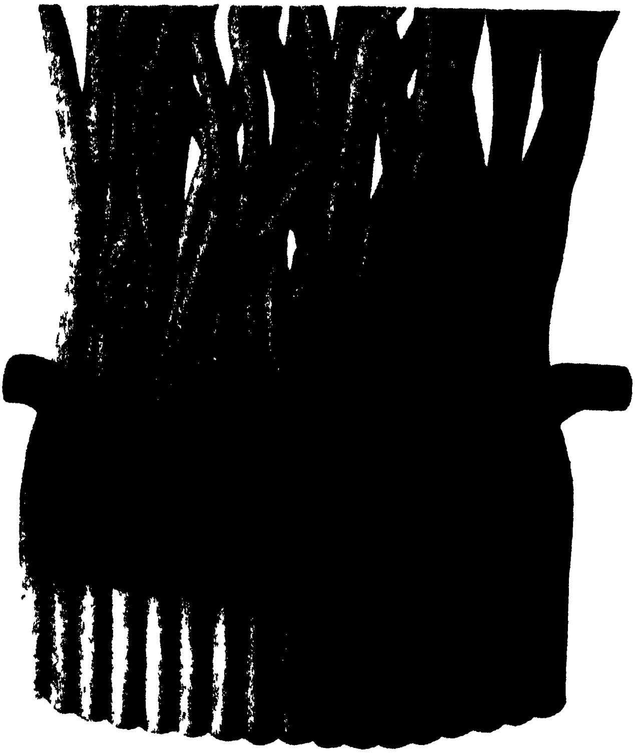

Fig. B. Basal portion of a loriculum of *Kofoidia loriculata* with a portion of the outer chromatic thread. Very greatly magnified.

and running to all parts of the cytoplasm; and (7) a series of fibers of two types running through the inner suspensory membrane from the collar to the nucleus, including a few larger, somewhat siderophile *major rhizoplast fibers* (*maj. rh. f.*) and numerous very fine, lightly eosinophile *minor rhizoplast fibers.*

Loricula.—These remarkable organelles consisting of cylindrical bundles of permanently integrated flagella, for which is proposed the name *loricula,* seem to present the climax of complexity of motor structure among the flagellates. Each is from 2 to 4.5 microns in diameter and consists of from 10 to 40, usually about 30, fairly large flagella about 100 microns in length, i.e., about four-fifths as long as the diameter of the body (pl. 23, fig. 1). In life, under normal conditions, the flagella of a loriculum are integrated to the tips (pl. 23, figs. 1, 2). In artificial media, and even more in fixed material, extensive distal fimbriation appears almost at once (pl. 23, fig. 5), but the basal integration is not disturbed save by maceration. Even when macerated the loricula separated from the body are intact and do not fall into individual flagella when torn apart. Each loriculum is sunk for about four microns in the cytoplasm (fig. G). A basal portion termed here the basal plate (*bas. pl.,* fig. D), from one to two microns in length, consists of the basal portions of the flagella. These show structural characters and staining reactions quite different from their distal portions. The basal plate is ordinarily unstained or takes a plasma stain, while with proper treatment the remainder of the bundle stains blue with haematoxylin. The basal portions of the flagella which constitute the basal plate are apparently rigid and perfectly cylindrical (fig. B). In life under high magnification the basal plate is readily seen as a bundle of highly refractive, closely approximated, short, rigid, cylindrical, parallel rods agreeing perfectly with the picture in fixed material (fig. B).

The basal plates of the loricula project internally from the granular cytoplasm in which they are imbedded and are seen to rest freely on, but not in, the material of a perfectly transparent, apparently structureless, clear zone (pl. 23, fig. 2). On contraction the whole base of the loriculum moves although it retains its general position, and the granular cytoplasm in which the loricula are basally imbedded is thus kept continually in motion.

The loricular series, while sometimes approximating a half-circle (fig. C, 2), is far more commonly, and apparently typically, a short spiral of from one to two turns (pl. 24, fig. 6). The spiral is leiotropic, the inner or proximal end, termed here the left, is strongly incurved and usually most anterior, while the outer or right end is usually straight and somewhat posterior.

The movement of a loriculum, illustrated in figure G (see p. 479), is rather in the nature of a pushing movement than a stroke or beat and is executed too slowly to be an effective means of locomotion. The loricular series is made still less efficient as a locomotor apparatus by the fact that the loricula do not beat synchronously. A contraction wave seems to run along the series from the proximal (left) end usually affecting two or three contiguous loricula more or less synchronously (pl. 23, fig. 2).

Under certain conditions, not understood as yet, the organisms in artificial media undergo a remarkable introversion of the entire loricular series into the cytosome. There the loricula continue beating, kneading up the cytoplasm in a most violent manner (fig. E, 1–4), and pushing out the ectoplasm and pellicle before them in long slender projections (fig. E, 8) which disappear on the retraction of the loriculum. This phenomenon emphasizes the peculiar flexibility and elasticity of the outer layers of the organism, a fact brought out also by microdissection experiments (fig. F).

Chromatic collar.—When *Kofoidia* is killed in osmic acid the picture is the same as that in life (pl. 23, fig. 2) the loricular bases projecting into, or onto, a zone of clear, perfectly transparent, apparently entirely homogeneous cytoplasm. When living individuals or those previously killed in osmic acid are treated with acetic acid, even in dilute solution, there appears like magic in this apparently homogeneous zone a collar-shaped structure whose dense, highly refractive, hence yellowish-green, appearance sets it strongly in contrast to the luminous clearness of the area from which it arises ghost-like and in which it lies. Smears fixed on the coverglass in two per cent osmic acid, or in Flemming's without acetic and stained in iron haematoxylin, show the collar as a smaller, faintly siderophile, achromatic, or even eosinophile structure, but in the same position and relations as the chromatic collar when fixed in Schaudinn's or Bouin's (pl. 23, figs. 3, 4, 5; fig. D, *chr. col.*). Such material fixed in osmic but subjected, after washing out the osmic, to Schaudinn's with acetic, showed the collar as a conspicuously chromatic structure again.

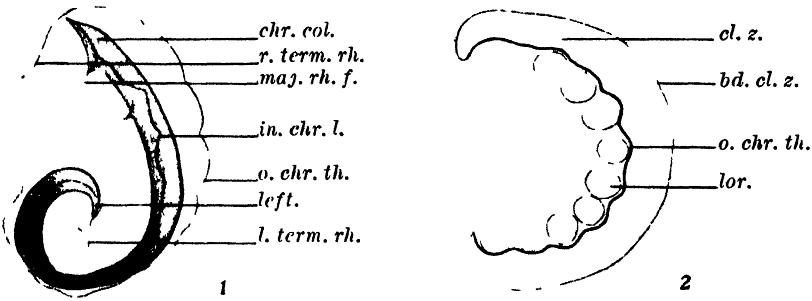

Fig. C. 1. Chromatic collar and outer chromatic line of *Kofoidia loriculata* as seen from above. × 1500.

Abbreviations: *chr. col.*, chromatic collar (right end); *in. chr. l.*, inner chromatic line; *left*, left end of chromatic collar; *l. term. rh.*, left terminal rhizoplast; *maj. rh. f.*, major rhizoplast fiber; *o. chr. th.*, outer chromatic thread; *r. term. rh.*, right terminal rhizoplast.

2. Diagram of semicircular loricular series with outer chromatic thread and clear zone. × 1500.

Abbreviations: *bd. cl. z.*, outer boundary of clear zone; *cl. z.*, clear zone; *lor.*, outline of a loriculum; *o. chr. th.*, outer chromatic thread.

This structure has been designated the *chromatic collar.* The term *collar* is used because its shape and appearance so obviously suggest this name, and *chromatic*, to distinguish it by a conspicuous characteristic from the entirely external structure termed collar in the choanoflagellates. By adopting this term we avoid the implications involved in the use of the term parabasal, which might seem obviously applicable to a conspicuously siderophile structure integrated to the neuromotor system and the nucleus. While it seems possible that this structure is homologous to the so-called parabasal apparatus of *Lophomonas* (Janicki, 1910, 1911) which Janicki originally termed the collar, the author is by no means convinced that this is the case. Nor is he convinced that these structures are homologous to the parabasal apparatus of other so-called hypermastigotes such as *Joenia, Joenina,* and *Parajoenia,* or to that of the polymastigotes.

This chromatic collar is usually band-shaped and uptilted like the rim of a saucer (pl. 24, fig. 15). It follows the loricular series throughout. The right end is ordinarily blunt but the left end is usually coiled and tapers to a slender tip (fig. C, 1; pl. 23, fig. 4). It presents a varying picture in stained material. It may appear dense, homogeneous, blue-black (fig. D, *chr. col.;* pl. 23, fig. 3; pl. 24, figs. 6, 8) or show varying intermediate conditions in which the proximal portion contains a dense chromatic band with granular chromatic masses in line with the bases of the loricula (pl. 23, fig. 5). Again, the chromatic areas may be smaller and discontinuous below the bases of the loricula in the proximal region; or the collar may show no internal chromatic areas but may appear hollow and show a chromatic line, the *internal chromatic line* (fig. C, 1, *in. chr. l.*), whose course again seems to follow the contour of the loricula. Finally, it may appear to be an entirely hollow structure, its surface alone taking the stain. Its upper margin, and more rarely the lower margin, show this same correspondence to the contours of the bases of the loricula (fig. A). This, together with the manner of appearance of the structure when brought out by acid, suggests the probability that the collar as seen in fixed material is a condensation of the structure which fills the clear zone in life.

Outer chromatic thread.—A small but conspicuously siderophile thread, the *outer chromatic thread* (*o. chr. th.*, figs. A, B, C, and D; pl. 23, figs. 4, 5; pl. 24, figs. 6, 7) lies closely applied to the outer surface of the loricular series and follows the contour of each loriculum (figs. A, B, D). This thread is continued in somewhat attenuated form to the nuclear membrane at either end. At the right end it joins the collar at or very near the tip (fig. H, 1) or lies along it (pl. 23, fig. 4; pl. 24, fig. 7), or, and perhaps more commonly, runs past it at a greater or less distance from it as a much less conspicuous thread, the *right terminal rhizoplast* (fig. C, 1), to end on the nuclear membrane (pl. 24, fig. 7). When it joins the collar, the latter gives off a fiber to the nucleus. This fiber we term also the right terminal rhizoplast, although in such cases it is probably in part the equivalent of one of the *major rhizoplast fibers* since here no such fibers are found near the tip of the collar.

The exact course of the left end of the chromatic thread has been more difficult to determine because of the incurvature of the end of the loricular series. In favorably oriented specimens it is seen to run around the tip of the collar, close to it but without contact with or attachment to it, to end on the nuclear membrane. This fiber connecting it to the nucleus has been designated the *left terminal rhizoplast* (fig. C). It is usually seen to be paralleled on its course to the nuclear membrane by a more or less conspicuous fiber which seems at times to represent a major rhizoplast fiber and at other times to be only the terminal line of the inner suspensory membrane (*maj. rh.*, fig. A).

The outer chromatic thread, while conspicuous in stained smears after acetic or picric fixation (figs. A, B, C, D; pl. 23, fig. 4, etc.), is entirely achromatic after osmic fixation nor has it ever definitely been made out in living individuals. The function of the outer chromatic thread is problematical. From its position it might be in the nature of a supporting structure (fig. B), but its relatively small size and its lack of tension seem to argue against this. Its siderophile staining reactions, its connections to the nucleus, and its intimate association with all the loricula suggest a neural function, perhaps that of conducting the impulses for the wave of contraction which runs along the loricula. From analogy with *Lophomonas* it would seem to represent a centroblepharoplast-rhizoplast complex.

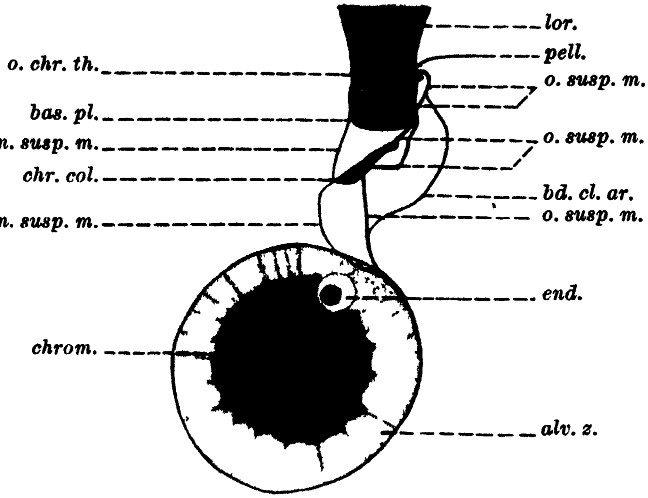

Fig. D. Base of one loriculum and optical section of the suspensorium, collar, and nucleus to show their relations. × 2,000.

Abbreviations: *alv. z.*, alveolar zone of nucleus; *bd. cl. ar.*, outer boundary of clear area; *bas. pl.*, basal plate of loriculum; *chrom.*, chromatin mass of nucleus; *chr. col.*, junction of inner suspensory membrane to chromatic collar, seen in optical section; *end.*, endosome in clear halo (note peripheral position and definite boundary of halo); *in. susp. m.*, inner suspensory membrane; *lor.*, loriculum; *pell.*, pellicle; *o. chr. th.*, outer chromatic thread; *o. susp. m.*, outer suspensory membrane.

Cytoendoskeleton.—The nucleus and neuromotor system of this flagellate are very firmly connected as is shown by the constant relations between them even in macerated specimens. Where dislocated or isolated, neuromotor systems are nearly always found to be accompanied by the nucleus. When this is not the case the fragmentation of the neuromotor system itself shows that it has been subjected to very violent treatment. This permanent connection seems due in large part, if not entirely, to two membranes, arising one along the inner margin of the chromatic collar and the other apparently along its outer margin. Both are attached to the nuclear membrane. These have been termed respectively the *outer suspensory membrane* and the *inner suspensory membrane* (figs. A and D; *o. susp. m.; in. susp. m.*). These follow the curvature of the collar and are attached internally to the nuclear membrane in the anterior half of its surface. The outer membrane is clearly to be made out covering the anterior surface of the outer chromatic thread (fig. A, fig. D) and in favorable preparations may be seen to run from the outer margin of the bases of the loricula, becoming thicker as it passes over the outer chromatic thread. Thence it passes to the collar inward, enclosing a very narrow space along the outer surface of the spiral (fig. D). It is usually attached again more or less broadly to the middle of the outer surface of the collar (fig. D) from which it curves out and posteriorly, and then in to the nuclear membrane at about its equator (fig. A). This con-

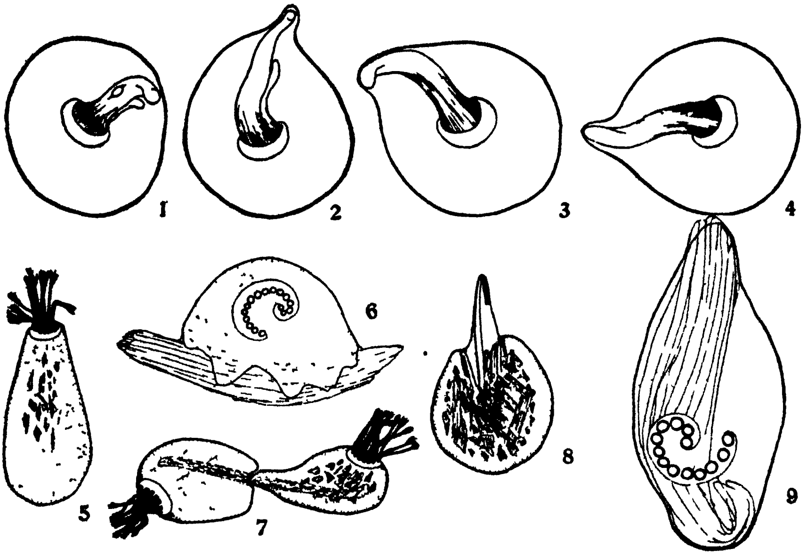

Fig. E. Sketches of *Kofoidia loriculata* from life.

1–4. Views of an individual with introverted loricula showing distortion of the cytosome due to the beating of loricula. The condition is not recent, and the loricula have become more or less attached to one another. × 400.

5. An individual showing the elongated form assumed at times. Note the position of the neuromotor system here as contrasted to its irregular position in individuals elongated by ingested masses. × 140.

6. An individual in process of ingesting a large wood mass. Loricula indicated by circles. × 200.

7. Two individuals attempting to ingest the same sliver of wood. × 150.

8. An individual in which the introversion of loricula is recent. One loriculum is shown extended, carrying pellicle and ectoplasm with it. Depressions at right and left indicate regions where corresponding loricula have just completed their retraction. × 220.

9. An individual which has practically completed the ingestion of a mass of filter paper fibers which greatly distend the body. × 300.

nection to the nucleus is loose or lacking in some individuals in stained smears, as is that to the collar (pl. 23, fig. 5). The inner surface of the membrane is often seen to be marked by granulations of considerable size (fig. A) and on several occasions a similar phenomenon has been observed in living individuals under pressure of a coverglass. Minute fibers may at times be seen running obliquely from the collar to the outer membrane (fig. A). This may be a further indication that the collar in life occupies a much larger portion of the area between outer and inner membranes. This seems indicated likewise by a clear area (figs. A and D) which lies outside the outer membrane and may well be due to a pulling inward of that membrane at its point of attachment to the collar when the contraction of the collar takes place during fixation. Arising from this outer membrane along its external surface as well as from its point of attachment to the nuclear membrane are many distinct achromatic fibers, termed *body filaments* (fig. A; fig. H, 1), which radiate to all parts of the cytosome.

The inner suspensory membrane is probably the more important of the two as regards the suspensory function. It shows up most distinctly after staining by the phosphotungstic acid haematoxylin method when it appears as a dense, comparatively thick canopy more or less shallowly bowl-shaped, its edges on the inner margin of the collar and broadly in contact and connection with the anterior surface of the nuclear membrane. In preparations fixed in Schaudinn's or Bouin's fluid and stained by the iron haematoxylin method, this membrane shows but faintly and usually appears tightly stretched between the collar and its nuclear attachment (fig. A). Through it run extremely slender, eosinophile fibers in a beautifully regular series converging toward the nuclear attachment, which we have designated *minor rhizoplast fibers* (fig. A). These are visible only under especially favorable conditions and hence show in but few preparations. Among these are usually one, two, or several fibers which are larger and somewhat siderophile, arising from very slender conical projections of the collar substance, which have been termed *major rhizoplast fibers* (fig. A; fig. C, 1; pl. 24, fig. 7). These fibers originate from the inner margin of the collar and the inner suspensory membrane itself is undoubtedly attached at that point. There are indications, however, that it is continued as a thin membrane along the inner sides of the intracytoplasmic bases of the loricula paralleling the similar continuation of the outer suspensory membrane along their outer surfaces (fig. D).

Nucleus.—The nucleus of the trophic stage of *Kofoidia* is of the vesicular type commonly found in the Hypermastigida with distinct nuclear membrane separated by a strikingly clear alveolar zone from a dense chromatin mass (figs. A, D, H, 1; pl. 23, figs. 4, 5; pl. 24, figs. 6–14). The clear zone is crossed by what appear to be slender chromatin threads from the central mass but which have been interpreted as the alveolar walls (fig. D). This general arrangement is found also in the prophases of mitosis (pl. 24, figs. 12, 14) and in the later telophases (fig. H, 2, 3).

The central chromatin mass is of the same general outline as the nucleus as a whole and is often so dense that it shows no structure (pl. 23, fig. 5; fig. D). This is the case in all prophase stages seen (pl. 24, figs. 12, 14). In most individuals in the trophic stage the chromatin mass presents a coarsely reticular appearance (fig. A; pl. 24, figs. 6, 7; pl. 23, fig. 4). In some individuals this may be seen to be purely superficial and to lie on the surface of a dense chromatin shell. This shell appears hollow within and contains one spherical endosome with a clear halo (pl. 24, fig. 8) and in some cases, in addition, a chromatin blob against the inner surface of the shell (pl. 24, fig. 11). Figures 9 and 11 of plate 24, represent a surface view and an optical section, respectively, of the same nucleus.

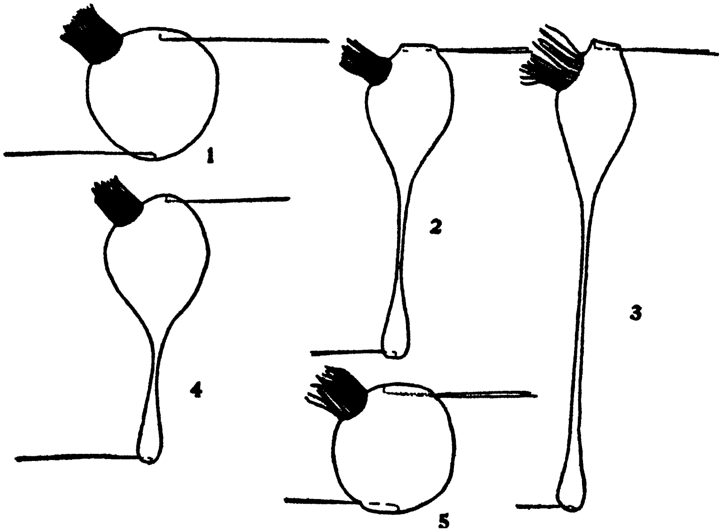

Fig. F. Diagrams to show flexibility and elasticity of pellicle and ectoplasm of *Kofoidia loriculata* as brought out by microdissection experiments. × 150.

The nucleus varies greatly in size. The individuals with small nuclei would seem to have recently divided, probably by multiple fission as indicated by the contrast in size between the nuclei in the telophase of first division (fig. H, 2) and those in the telophase of second division (fig. H, 3).

The affinity of the chromatin mass for the stain has made difficult the study of the nucleolar structures. One seems to be universally present. It usually stains less densely and is more translucent than the chromatin. Such a body has been seen emerging from the chromatin mass in mitosis (pl. 24, fig. 14) and seems to represent an endosome. Some individuals in what seems to be the trophic stage show what appears to be a similar body in a vacuole at the periphery of the chromatin mass. In the specimen shown in fig. 4 of plate 23, this body seems to be emerging from the chromatin mass.

Cytoplasm and pellicle.—The cytoplasm of *Kofoidia* ordinarily exhibits a granular structure after fixation in Schaudinn's or Bouin's fluid (fig. A) but a distinctly alveolar arrangement is sometimes apparent after fixation in osmic or Flemming's fluid and, rarely, in starved individuals fixed in Bouin's fluid (pl. 23, fig. 5). Aside from the structures of the cytoendoskeleton described in connection with the neuromotor system the cytoplasm exhibits no structural differentiations. While the granules are smaller and more crowded near the periphery no clear demarcation of ectoplasm and endoplasm has been made out although the wood or filter paper fragments which ordinarily crowd the cytosome tend to be absent in a peripheral zone. A study of living material shows that under normal conditions these wood particles occur up to the pellicle. In artificial media, however, either those near the periphery are thrown out in some manner not understood, or, where this does not occur, there seems to be a rearrangement of materials within the organism establishing a clear subpellicular zone which simulates an ectoplasm.

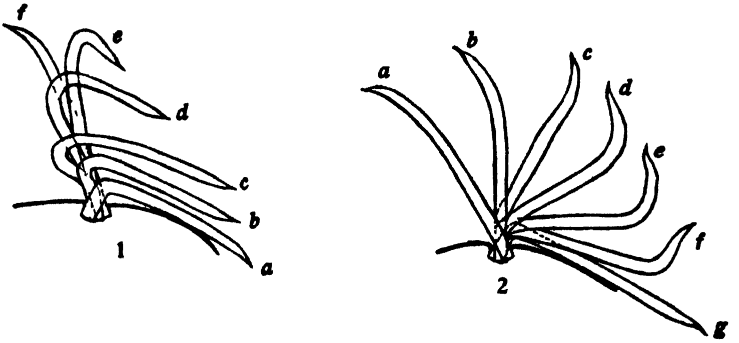

Fig. G. A diagrammatic representation of various phases in the loricular stroke. 1, The upstroke; 2, the downstroke. × 300.

The pellicle is thin but distinct and relatively strong. Its strength and elasticity are shown by the resistance it offers to the plunging efforts at escape of the introverted loricula (fig. E, 1–4, 8), and to the impacts of the tiny hypermastigote, *Microspirotrichonympha,* present in large numbers in the intestine of *K. simplicicornis,* and by its reactions under microdissection (fig. F). The pellicle shows up most clearly in material fixed in osmic where it is seen to be perfectly homogeneous in optical section. It is seen to wrinkle and break in many preparations, thus giving an impression of rigidity. If the pellicle is rigid, the organism is, at times, able to render it flexible, to resorb it, or in some way to allow for the taking in of relatively large masses of food (fig. E, 6, 9), and to regenerate it after ingestion and also after the introversion of the loricula.

The surface of the pellicle is covered with small rod-shaped bacteria. Some rods present the appearance of being in the pellicle. Whether these are simply closely applied bacteria or rod-shaped pellicular reinforcements such as found in *Lophomonas* (Janicki, 1910), *Devescovina* (Janicki, 1915), and *Staurojoenina* (Kirby, 1926), which also have been recently described as bacteria by Grassé (1926), the author was unable to determine.

MULTIPLICATION

Material is lacking for working out of the details of the process of mitosis and fission in *Kofoidia.* The process so far as known agrees with that in *Lophomonas.* Preliminary to mitosis the nucleus deserts the old motor system (pl. 24, fig. 13). The deserted motor organelles degenerate after a considerable period of independent activity (fig. H, 2, 3). The new neuromotor organelles arise as outgrowths from the ends of the paradesmose, which thus seem to function as centrosomes although these cannot be identified as separate structures (pl. 24, figs. 11–14, 10; fig. H, 2, 3).

The question at once arises as to the origin of the paradesmose and the fate of the collar and outer chromatic thread, since these latter are not to be seen in the deserted neuromotor systems (fig. H, 2, 3; pl. 24, fig. 13). If the author's surmise that the collar is merely a condensation of the structure of the clear zone be correct, it is to be expected that the tearing away of the nucleus to which it is so intimately bound would be preceded or accompanied by a breaking down of the structure of the clear zone. The outer chromatic thread is not found remaining with the deserted motor organelles. It holds the relations to the motor organelles and the nucleus, respectively, which would characterize a rhizoplast-centroblepharoplast complex in other flagellates. Moreover, as will be brought out later, a similar structure seems connected with the appearance of the paradesmose in *Lophomonas.* I therefore venture to predict that material showing preliminary stages in the migration of the nucleus will show the outer chromatic thread condensing to its nuclear connections, the terminal rhizophasts, to form the paradesmose. The paradesmose must increase rapidly in size since it is much more massive in early telophase (pl. 24, fig. 10) than in prophase (pl. 24, figs. 12, 14).

The loricula of the new series arise entirely within the cytoplasm (pl. 24, fig. 13; fig. II, 2) and are apparently not exserted until fully formed, or nearly so, since no individuals with unusually small loricula were encountered. Multiple fission occurs (fig. H, 3).

Genus **Kofoidia** gen. nov.

Diagnosis.—Xylophagous lophomonads with massive, globular cytosomes bearing an essentially spiral neuromotor system concentrated at one end and characterized by a series of cylindrical, permanently integrated, flagellar bundles called loricula. These rest in life upon a clear hyaline zone in or from which there appears on fixation or death a dense band, the chromatic collar. A siderophile thread runs around the bases of the loricula just previous to their emergence from the cytoplasm and connects to the nuclear membrane at both ends. The neuromotor spiral is joined to the nucleus along its length by an outer and an inner suspensory membrane between which lies the clear zone in life and the chromatic collar in fixed individuals. From the outer suspensory membrane supporting fibers run through the cytosome. Through the inner suspensory membrane slender fibers run to the nuclear membrane. The nucleus is large, vesicular, with a peripheral

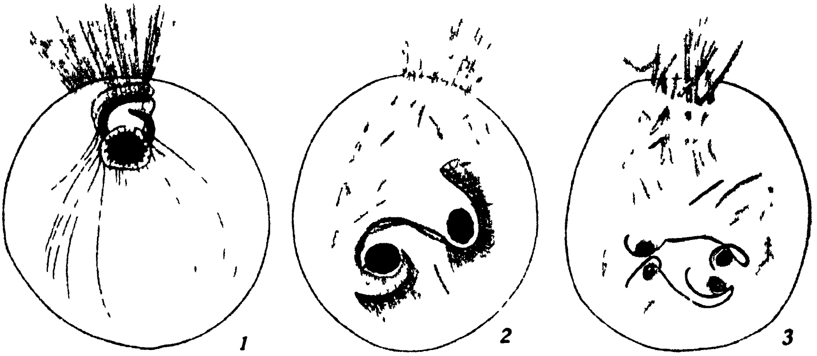

Fig. II. *Kofoidia loriculata* from smears.

1. A stained individual seen from the right side with the animal tipped away from the observer so as to throw the neuromotor system somewhat below. Note the clear distinction between the straight right end of the collar (here above) and the incurved left end (here below). In this individual the outer chromatic line clearly joins the collar before running to the nucleus as the right terminal rhizoplast which here probably is united with a major rhizoplast fiber. The cytoplasm is omitted but the body filaments indicated. × 250.

2. An early telophase. Note somewhat damaged but still functional loricula without other remnants of the old neuromotor system; at other end of body note large paradesmose, in this case plainly double and each part composed of twisted fibers; at either end of paradesmose are the new neuromotor systems and nuclei. Wood fragments shown but cytoplasm not indicated. × 250.

3. Multiple fission. A double telophase. Loricula and outer chromatic threads of neuromotor systems not shown, actually as in figure 2 but smaller. Note that the two telophase sets *as wholes* are mirror images of each other, the posterior one being deeper and the anterior one higher in the cytoplasm. × 250.

alveolar zone and a central heavy chromatin meshwork containing one or two large siderophilic endosomes in clear halos. At mitosis the nucleus leaves the old organelles which remain active for some time but gradually disintegrate. The new neuromotor elements all arise *de novo* from the ends of the paradesmose *within the cytoplasm.* Multiple fission occurs.

Type species.—Kofoidia loriculata sp. nov. from the intestine of the termite, *Kalotermes simplicicornis* Banks, from Texas, Arizona, California, and Lower California.

OCCURRENCE

Kofoidia loriculata has been found present in large numbers in the hind intestine of all normal nymphs and soldiers of *Kalotermes simplicicornis* Banks which we have examined. It is found throughout the gut from the enlarged region near the point of origin of the Malpighian tubules to the pre-rectal region. They are usually seen in groups and are conspicuous within the intestine even under low magnification because of their relatively large size and their massive globular shape. Both of these characters are emphasized by the opacity and the yellow or light brown color, due to the crowded wood fragments which ordinarily fill the cytosome (fig. A). The constant activity of the long, tentacle-like loricula (pl. 23, fig. 1) also draws the observer's attention. Several of the other species of flagellates which inhabit the intestine of this termite are present in much greater numbers, but in protoplasmic mass *K. loriculata* seems to be the dominant element and the relatively enormous amount of wood which it ingests points to it as playing the leading rôle in cellulose digestion in this termite.

SIGNIFICANCE OF CONSTANT MOTOR ACTIVITY

It seems worth while at this time to consider the significance of the constant activity of the motor organelles of the unattached intestinal flagellates, particularly of those of the termite intestine. The necessity of such activity on the part of saprozoic, non-xylophagous species, whether attached or free, is obvious since they are dependent for food supply on materials absorbed from the intestinal fluid. That such activity is not necessary for obtaining food *particles,* however, is demonstrated by attached, xylophagous forms such as *Oxymonas* Janicki (Kofoid and Swezy, 1926*a*), *Proboscidiella* (Kofoid and Swezy, 1926*b*), and others of similar habit as yet undescribed, which are characterized by negligible flagellar equipment and activity and yet show by their great numbers and by their wood-filled cytosomes convincing evidence of a successful holozoic habit.

There remains the obvious correlation between a free habit in the intestinal lumen on the one hand and on the other the constant activity of motor organelles together with the high complexity of these organelles which we would seem justified in connecting with their constant activity. This constant activity of the motor organelles does not necessarily involve a rapid or constant progression. On the contrary, *Kofoidia,* for example, is practically stationary in habit. Why then the extremely complex neuromotor system and constantly active motor organelles? The author believes the constant motor activity to represent a negative rheotropic reaction to the constant, even though mild, pressure toward the outside due to the peristaltic movements of the termite intestine against which these organisms have had to contend. The conception that these organisms have evolved under the constant necessity, throughout the millions of years during which they have presumably existed within the termite gut, of maintaining themselves against the slow but persistent current toward the anal orifice and the outside, to respond passively to which would mean extinction, seems by far the most satisfying explanation of the development of such a bewildering array of the most complex neuromotor systems found among the flagellates. The emphasis would seem to have been not so much on strikingly effective locomotor systems, although such have resulted in certain cases, as on constantly active and therefore highly developed motor systems, the ability of the organism to maintain itself against the peristaltic current being the *sine qua non* of existence in the environment. This gives at least a reasonable explanation of the condition which we find on opening the intestine of any infested termite—fixed forms with little if any development or activity of motor organelles, save when of saprozoic habit, as contrasted with the universality, so far as our present knowledge goes, of constant activity of motor organs on the part of *all free* Protozoa of the termite intestine whether xylophagous or non-xylophagous.

Systematic Position

Kofoidia presents no special affinities with any of the members of the order Hypermastigida as heretofore constituted, save with *Lophomonas.* The shape, the nature, the location and arrangement of the neuromotor organelles and cytoendoskeleton, their relations to the nucleus during the trophic stage, and particularly the behavior during fission and multiplication, all point very definitely to relationship with *Lophomonas.*

Since the above was written there has appeared an article by Kudo on *Lophomonas blattarum* (1926) and more recently it has been my privilege to examine material of both *L. blattarum* and *L. striatum* prepared by J. F. Lamiman, working under the direction of Professor Kofoid. Mr. Lamiman has kindly given me permission to announce that a preliminary survey seems to emphasize the affinity which I had predicted on the basis of Janicki's work (1910).

The *Kelch,* which is obviously homologous with the suspensorium of *Kofoidia,* is certainly not a continuation of the flagella as Kudo figures and describes it but may plainly be seen flaring near the bases of the tentacles as Janicki figured it, like the margin of a goblet. Apparently in it, at its anterior end between outer and inner membranes, is an outer chromatic thread probably the distal zone of basal granules of Janicki. This would seem to be the "blepharoplast ring" of some of Kudo's figures, particularly in individuals in which mitosis is about to be initiated but which he confused with the row of basal blepharoplasts on the flagella. He says that "one end of the blepharoplast-ring bends down and becomes attached to the anterior region of the nucleus" which part becomes detached and later gives rise to the paradesmose. When we consider that he believes his "blepharoplast-ring" to be no more than a row of granules at the bases of the flagella and that in the figures showing this phenomenon there is no derangement of the flagella, the explanation plainly limps. But the matter becomes quite clear when we have an external continuous band (the outer chromatic thread) which contains, or functions as, a centrosome and by homology, in the case of *Kofoidia,* as a blepharoplast-rhizoplast complex. In *Kofoidia* the basal portions of the flagella present staining reactions and structural characters quite other than those characteristic of blepharoplasts (fig. B) and these basal portions while contiguous in each loriculum (forming the so-called basal plate) show no apparent rhizoplast connections even within the loriculum and certainly none between the loricula. It seems probable that the coordinating function has been taken over by the outer chromatic thread whatever may be the nature of the basal granules.

The author is inclined to believe that careful study will show the *Kelch* to represent the suspensorium as a whole with outer and inner membranes close together. This brings the axostyle fibers into agreement with the body filaments of *Kofoidia* but leaves the so-called parabasal of *Lophomonas* without homologue in *Kofoidia.* It also suggests the possibility that the so-called basal granules of *Lophomonas* are in reality below the ends of the flagella and represent the homologue of the chromatic collar of *Kofoidia.* Such a condition might account for some of the heavy, independently bent, chromatic bands depicted by Kudo. The possibility remains, of course, that both here and in *Kofoidia* the collar forms the paradesmose.

Whatever the final decision as to the details of their homologies, there can be no doubt as to the relationship of *Lophomonas* and *Kofoidia.* The possession of so distinctly unique and highly complex a structure as the loriculum, however, together with other differences, makes necessary the erection of a new family to receive the peculiar genus *Kofoidia.*

Family KOFOIDIIDAE fam. nov.

Diagnosis.—Lophomonads characterized by locomotor organelles in the form of a spirally arranged series of cylindrical bundles of permanently integrated flagella, the loricula, resting upon a clear, hyaline substance in which appears, on fixation, a chromatic band, the chromatic collar. Nucleus and loricula are integrated by a chromatic thread. Motor organelles and nucleus are held together by a suspensorium from which cytoendoskeletal filaments radiate through the cytosome.

Type genus.—*Kofoidia* gen. nov.

The two families Lophomonadidae and Kofoidiidae differ so widely in structure and methods of reproduction from all other hypermastigotes as to indicate once more, as was suggested by the study of *Hoplonympha* (Light, 1926*a*) and *Metadevescovina* (Light, 1926*b*), the polyphyletic nature of the order Hypermastigida as now constituted.

The time would seem to have come for undertaking a preliminary division of that order, as now constituted, into groups more accurately representative of phylogenetic relationships as expressed in structure and reproductive processes.

There has been no clear indication of any type family for the order Hypermastiginia. The name, more properly Hypermastigida (Calkins, 1926), has been associated most closely in the literature with the *Trichonympha* group. The author proposes therefore to restrict this order to the fairly homogeneous group of families including the Trichonymphidae Grassi, the Holomastigotidae Grassi, probably the Teratonymphidae Koidzumi and the Spirotrichonymphidae Grassi (although the latter has an axostyle-like structure), and possibly the Staurojoeninidae Grassi and the Hoplonymphidae Light, although the last two would certainly constitute a suborder at least and perhaps a separate order.

This leaves three families to be redistributed, the Lophomonadidae Janicki, the Kofoidiidae fam. nov., and the Joeniidae Janicki. The Joeniidae, although poorly known and badly in need of reinvestigation, present many plain affinities to the Polymastigida and are hereby provisionally assigned to that order as redefined by the author (1926*b*) until such time as a careful revision permits fuller knowledge of the true affinities of all forms there included.

For the Lophomonadidae and Kofoidiidae the author proposes the erection of a new order, the Lophomonadida, whose affinities are probably more clearly with the Polymastigida than with the Hypermastigida as here constituted.

Order LOPHOMONADIDA ord. nov.

Characters.—Mastigophora inhabiting the intestines of cockroaches and termites, whose numerous flagella, separate or in bundles (loricula), are arranged in a spiral or broken circle at one end of the cytosome and whose neuromotor system is integrated to the nucleus by a cytoendoskeleton from which supporting filaments project posteriorly into the cytosome.

In multiplication the nucleus deserts the old motor apparatus, which continues to function for some time but ultimately disintegrates, and the new neuromotor apparatuses arise from the ends of the paradesmose.

Multiple fission occurs resulting in individuals with a number of functioning enucleate neuromotor systems and a number of functioning nucleate systems together with others not as yet functional and lying entirely within the cytoplasm.

Type family.—Lophomonadidae, Janicki.

The order to include at the present time the families Lophomonadidae and Kofoidiidae.

SUMMARY

Kofoidia loriculata described here and *Hoplonympha natator* described in a previous paper (Light, 1926*a*) are in contrast in habit, method of binary fission, and structurally in all save fundamental flagellate characters and in the possession of many flagella; this in spite of the presumably tremendous period of time during which they have been associated in the intestine of the same termite species.

Kofoidia shows unmistakable relations to *Lophomonas* of the cockroaches, which, although the nearest insect relatives of the termites, were well differentiated before the *Mesozoic*. The differences between *Kofoidia* and *Hoplonympha* have probably, therefore, a genetic basis in the origin of the two organisms from stocks already differentiated at the time of infection of the termite.

Kofoidia is characterized by (1) a massive, spherical body, usually crowded with wood, crowned by a short spiral row of eight to sixteen loricula (permanently integrated, cylindrical bundles of flagella)—a type of locomotor organelle new for the flagellates, very suggestive of the cirrus of the ciliates; (2) a dense hyaline supporting structure underlying these loricula which shrinks to form, or in which there becomes apparent on fixation, the chromatic collar; (3) a conspicuous siderophile thread, the outer chromatic thread, probably a centro-blepharoplast-rhizoplast complex, which runs along the outer surface of the loricular series and is connected at both ends to the nucleus; and (4) a cytoendoskeleton in the form of a suspensorium connecting the neuromotor spiral throughout its course to the nuclear membrane, through the inner membrane of which run numerous fine fibers connecting collar and nucleus, and from the outer membrane of which run body filaments to all parts of the cytoplasm, representing, perhaps, a dispersed axostyle.

Although *Kofoidia* is sluggish and usually non-locomotor, its loricula maintain a constant activity, as do the motor organs of all free flagellates of the termite intestine. The author believes the constant activity of the motor organs of the free flagellates of termites to be the expression of a negative rheotropic reaction to the peristaltic pressure of the termite intestine. The age-long necessity for such motor activity is suggested as the most potent factor in the evolution of these multiflagellate Mastigophora resulting in the development of neuromotor systems far more complex than are found in any other flagellates.

Preliminary to binary fission the nucleus of *Kofoidia* deserts the old neuromotor system, which continues to function for a time, then migrates to the other end of the cell where the mitotic process takes place. Multiple fission occurs.

The paradesmose, which probably takes its origin from the outer chromatic thread, gives rise at its ends to the two sets of neuromotor structures.

A new family, the Kofoidiidae, is erected with the new genus *Kofoidia* as its type genus. The new family is most nearly related to the Lophomonadidae Janicki and but distantly if at all to the other families of the order Hypermastigida as at present constituted.

The author proposes the subdivision of the clearly polyphyletic order Hypermastigida, reserving that name for the homogeneous group which includes the families, Trichonymphidae Grassi, Holomastigotidae Grassi, Spirotrichonymphidae Grassi, Teratonymphidae Koidzumi, and probably the Staurojoeninidae Grassi and Hoplonymphidae Light. Owing to the fact that in every fundamental character they are distinct from the above, a new order, the Lophomonadida, is erected for the two closely related families Lophomonadidae Janicki and Kofoidiidae fam. nov. That Lophomonadida is more closely related to Polymastigida (as redefined by Light, 1926*b*) than to the Hypermastigida thus restricted, seems indicated by their axostyle-like structures. Some, if not all, of the organisms in the family Joeniidae belong in the order Polymastigida as redefined, and the family is there assigned awaiting a much needed revision.

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EXPLANATION OF PLATES

All figures of *Kofoidia loriculata* gen. nov., sp. nov., from *Kalotermes simplicicornis* Banks. Camera lucida drawings or microphotographs from living individuals or from smears fixed in Bouin's or Schaudinn's fluid and stained by the iron haematoxylin method.

PLATE 23

Fig. 1. A living individual represented as opaque. Cytosome represented smaller in proportion to loricula than is usual. × 350.

Fig. 2. A living individual represented as transparent. Note loricular bases within the cytoplasm and resting on the clear zone. A few of the many fragments of wood which crowd the cytosome are represented. × 350.

Fig. 3. Photomicrograph of an individual from a stained smear, with dorsal surface up. Note darker basal plates of loricula, very dark chromatic collar and nucleus, and the wood fragments massed near the lateral and posterior regions of the nucleus. × 120.

Fig. 4. Collar, outer chromatic line and nucleus of an individual in smear preparation. Note endosome apparently leaving vacuole whose wall joins nuclear membrane. × 600.

Fig. 5. An individual from a stained smear made from the intestine of a termite starved because of refusal to adopt filter paper diet. The right side is up and the incurved left end of the collar is visible. Note small, clear cytosome showing distinctly alveolar cytoplasm. × 600.

3

4

PLATE 24

Fig. 6. Neuromotor system seen from above. Note outer chromatic thread apparently joining collar at either end. Loricula indicated by basal optical sections. × 1300.

Fig. 7. Nucleus and right half of collar and loricular series, loricula represented as cut off near bases. Note lighter basal plates of loricula, outer chromatic thread which runs along right tip of collar to pass to nucleus as right terminal rhizoplast, collar containing inner chromatic line conforming to bases of loricula and a major rhizoplast fiber from collar near tip to nucleus. × 1300.

Fig. 8. Collar and nucleus of the small type. Note that collar is rather blunt and not incurved at either end. Nucleus shown in optical section bringing out the hollow nature of the chromatin mass with the endosome within it. See also fig. 11 for this. × 1300.

Fig. 9. Same nucleus as in fig. 11 but with chromatin mass shown in surface view showing the rib-like arrangement of the chromatin on the shell. × 1300.

Fig. 10. A photomicrograph of a very early telophase stage showing the massive twisted paradesmose which seems to push apart the daughter nuclei in which its ends lie in notches. At either end the lines of the flagella may be made out.

Fig. 11. An optical section of the nucleus shown in fig. 9 showing the shell of chromatin containing the endosomes. × 1300.

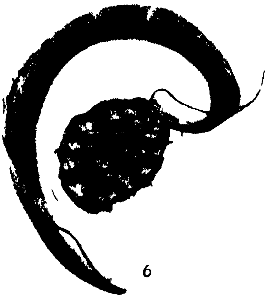
Fig. 12. A nucleus in prophase (from the individual shown in figure 13) showing the neuromotor elements already well developed and within the cytoplasm. × 1300.

Fig. 13. Outline diagram of relative positions of deserted motor organs and prophase nucleus. × 400.

Fig. 14. A well differentiated nucleus in early prophase showing the large, corded, band-like paradesmose, the absence of anything clearly in the nature of chromosomes and the persistent endosome. × 2100.

Fig. 15. Collar seen from above showing bifurcated appearance of right tip often seen in individuals in which the outer chromatic thread joins the upper margin of tip and the terminal rhizoplast takes origin from its lower margin. × 1300.

Fig. 16. A collar seen through the cytosome showing incurved left end (at right). × 1300.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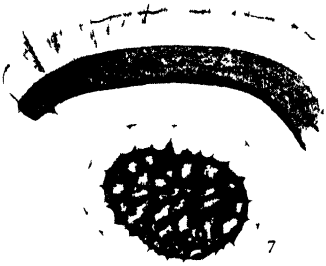
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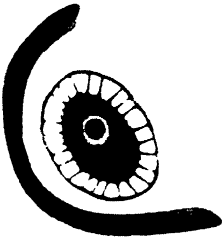
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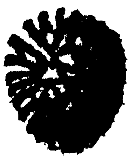
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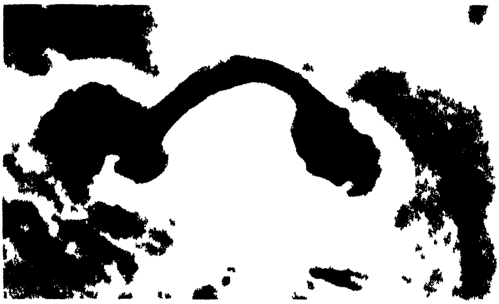
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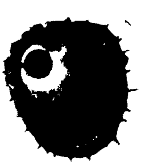
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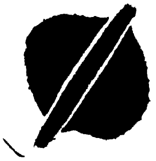
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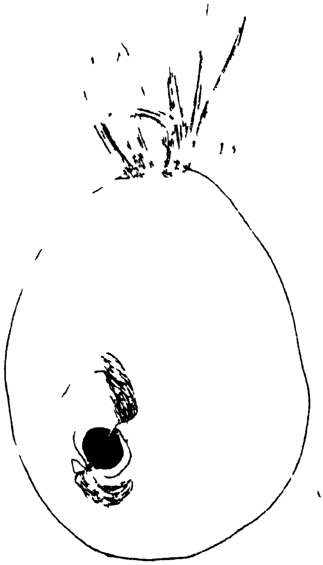
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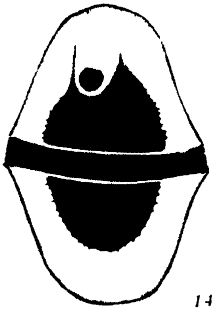
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ERRATA

Page 313, labeling of fig. 4. *For* sympatheticus *read* sympathicus.